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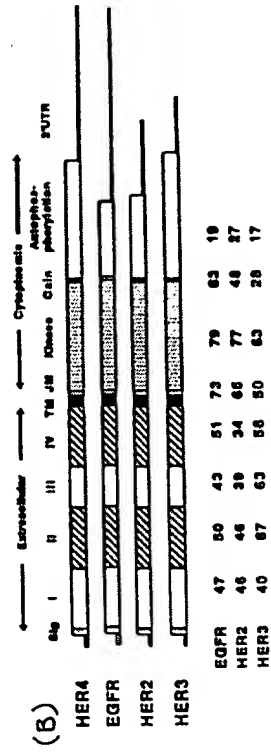
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HER4, a human receptor tyrosine kinase of the epidermal growth factor receptor family.

The molecular cloning, expression, and biological characteristics of a novel receptor tyrosine kinase related to the epidermal growth factor receptor, termed HER4/p180^{erbB4}, are described. A HER4 ligand capable of inducing cellular differentiation of breast cancer cells is also disclosed. In view of the expression of HER4 in several human cancers and in certain tissues of neuronal and muscular origin, various diagnostic and therapeutic uses of HER4-derived and HER4-related biological compositions are provided.

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FIGURE 3



1. INTRODUCTION

The present invention is generally directed to a novel receptor tyrosine kinase related to the epidermal growth factor receptor, termed HER4/p180^{erbB4} ("HER4"), and to novel diagnostic and therapeutic compositions comprising HER4-derived or HER4-related biological components. The invention is based in part upon applicants discovery of human HER4, its complete nucleotide coding sequence, and functional properties of the HER4 receptor protein. More specifically, the invention is directed to HER4 biologics comprising, for example, polynucleotide molecules encoding HER4, HER4 polypeptides, anti-HER4 antibodies which recognize epitopes of HER4 polypeptides, ligands which interact with HER4, and diagnostic and therapeutic compositions and methods based fundamentally upon such molecules. In view of the expression of HER4 in several human cancers and in certain tissues of neuronal and muscular origin, the present invention provides a framework upon which effective biological therapies may be designed. The invention is hereinafter described in detail, in part by way of experimental examples specifically illustrating various aspects of the invention and particular embodiments thereof.

2. BACKGROUND OF THE INVENTION

Cells of virtually all tissue types express transmembrane receptor molecules with intrinsic tyrosine kinase activity through which various growth and differentiation factors mediate a range of biological effects (reviewed in Aaronson, 1991, *Science* 254: 1148-52). Included in this group of receptor tyrosine kinases (RTKs) are the receptors for polypeptide growth factors such as epidermal growth factor (EGF), insulin, platelet-derived growth factor (PDGF), neurotrophins (i.e., NGF), and fibroblast growth factor (FGF). Recently, the ligands for several previously-characterized receptors have been identified, including ligands for c-kit (steel factor), met (hepatocyte growth factor), trk (nerve growth factor) (see, respectively, Zsebo et al., 1990, *Cell* 63: 195-201; Bottardo et al., 1991, *Science* 251: 802-04; Kaplan et al., 1991, *Nature* 350: 158-160). In addition, the soluble factor NDF, or heregulin-alpha (HRG- α), has been identified as the ligand for HER2, a receptor which is highly related to HER4 (Wen et al., 1992, *Cell* 69:559-72; Holmes et al., 1992 *Science* 256:1205-10). However, at present, the ligands for a number of isolated and/or characterized receptor tyrosine kinases have still not been identified, including those for the eph, eck, elk, ret, and HER3 receptors.

Biological relationships between various human malignancies and genetic aberrations in growth factor-receptor tyrosine kinase signal pathways are known to exist. Among the most notable such relationships involve the EGF receptor (EGFR) family of receptor tyrosine kinases (see Aaronson, *supra*). Three human EGFR-family members have been identified and are known to those skilled in the art: EGFR, HER2/p185^{erbB2}, and HER3/p160^{erbB3} (see, respectively, Ullrich et al, 1984, *Nature* 309: 418-25; Coussens et al., 1985, *Science* 230: 1132-39; and Plowman et al., 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87: 4905-09). EGFR-related molecules from other species have also been identified.

The complete nucleotide coding sequence of other EGFR-family members has also been determined from other organisms including: the drosophila EGFR ("DER": Livneh, E. et al., 1985, *Cell* 40: 599-607), nematode EGFR ("let-23": Aroian, R.V. et al., 1990, *Nature* 348: 693-698), chicken EGFR ("CER": Lax, I. et al., 1988, *Mol. Cell. Biol.* 8: 1970-1978), rat EGFR (Petch, L.A. et al., 1990, *Mol. Cell. Biol.* 10: 2973-2982), rat HER2/neu (Bargmann, C.I. et al., 1988, *Nature*, 319: 226-230) and a novel member isolated from the fish and termed *Xiphophorus* melanoma related kinase ("Xmrk": Wittbrodt, J. et al., 1989, *Nature* 342: 415-421). In addition, PCR technology has led to the isolation of other short DNA fragments that may encode novel receptors or may represent species-specific homologs of known receptors. One recent example is the isolation tyro-2 (Lai, C. and Lemke, G., 1991, *Neuron* 6: 891-704) a fragment encoding 54 amino acids that is most related to the EGFR family.

Overexpression of EGFR-family receptors is frequently observed in a variety of aggressive human epithelial carcinomas. In particular, increased expression of EGFR is associated with more aggressive carcinomas of the breast, bladder, lung and stomach (see, for example, Neal et al., 1985, *Lancet* 1: 368-68; Sainsbury et al., 1987, *Lancet* 1: 1398-1402; Yasui et al., 1988, *Int. J. Cancer* 41: 211-17; Veale et al., 1987, *Cancer* 55: 513-18). In addition, amplification and overexpression of HER2 has been associated with a wide variety of human malignancies, particularly breast and ovarian carcinomas, for which a strong correlation between HER2 overexpression and poor clinical prognosis and/or increased relapse probability have been established (see, for example, Slamon et al., 1987, *Science* 235: 177-82, and 1989, *Science* 244: 707-12). Overexpression of HER2 has also been correlated with other human carcinomas, including carcinoma of the stomach, endometrium, salivary gland, bladder, and lung (Yokota et al., 1986, *Lancet* 1: 765-67; Fukushima et al., 1986, *Mol. Cell. Biol.* 6: 955-58; Yonemura et al., 1991, *Cancer Res.* 51: 1034; Weiner et al., 1990, *Cancer Res.* 50: 421-25; Geurin et al., 1988, *Oncogene Res.* 3:21-31; Semba et al., 1985, *Proc. Natl. Acad.*

Sci. U.S.A. 82: 6487-6501; Zhau et al., 1990, Mol. Carcinog. 3: 354-57; McCann et al., 1990, Cancer 65: 88-82). Most recently, a potential link between HER2 overexpression and gastric carcinoma has been reported (Jaehne et al., 1992, J. Cancer Res. Clin. Oncol. 118: 474-78). Finally, amplified expression of the recently described HER3 receptor has been observed in a wide variety of human adenocarcinomas (Poller et al., 1992, J. Path., in press; Krause et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86: 9193-97; European Patent Application No. 91301737, published 9.4.91, EP 444 961).

Several structurally related soluble polypeptides capable of specifically binding to EGFR have been identified and characterized, including EGF, transforming growth factor- α (TGF- α), amphiregulin (AR), heparin-binding EGF (HB-EGF), and vaccinia virus growth factor (VGF) (see, respectively, Savage et al., 1972, J. Biol. Chem. 247: 7812-21; Marquardt et al., 1984, Science 223: 1079-82; Shoyab et al., 1989, Science 243: 1074-78; Higashiyama et al., 1991, Science 251: 836-39; Twardzik et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82: 5300-04). Despite the close structural relationships among receptors of the EGFR-family, none of these ligands has been conclusively shown to interact with HER2 or HER3.

Recently, several groups have reported the identification of specific ligands for HER2. Some of these ligands, such as gp30 (Lupu et al., 1990, Science 249: 1552-55; Bacus et al., 1992, Cell Growth and Differentiation 3: 401-11) interact with both EGFR and HER2, while others are reported to bind specifically to HER2 (Wen et al., 1992, Cell 69: 559-72; Peles et al., 1992, Cell 69: 205-18; Holmes et al., 1992, Science 258: 1205-10; Lupu et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89: 2287-91; Huang et al., 1992, J. Biol. Chem. 267: 11508-121). The best characterized of these ligands are neu differentiation factor (NDF) purified and cloned from ras-transformed Rat1-EJ cells (Wen et al., Peles et al., *supra*), and the heregulins (HRF- α , - β 1, - β 2, - β 3), purified and cloned from human MDA-MB-231 cells (Holmes et al., *supra*). NDF and HRG- α share 93% sequence identity and appear to be the rat and human homologs of the same protein. Both of these proteins are similar size (44-45 kDa), increase tyrosine phosphorylation of HER2 in MDA-MB-453 cells and not the EGF-receptor, and have been reported to bind to HER2 in cross-linking studies on human breast cancer cells. In addition, NDF has been shown to induce differentiation of human mammary tumor cells to milk-producing, growth-arrested cells, whereas the heregulin family have been reported to stimulate proliferation of cultured human breast cancers cell monolayers.

The means by which receptor polypeptides transduce regulatory signals in response to ligand binding is not fully understood, and continues to be the subject of intensive investigation. However, important components of the process have been uncovered, including the understanding that phosphorylation of and by cell surface receptors hold fundamental roles in signal transduction. In addition to the involvement of phosphorylation in the signal process, the intracellular phenomena of receptor dimerization and receptor crosstalk function as primary components of the circuit through which ligand binding triggers a resulting cellular response. Ligand binding to transmembrane receptor tyrosine kinases induces receptor dimerization, leading to activation of kinase function through the interaction of adjacent cytoplasmic domains. Receptor crosstalk refers to intracellular communication between two or more proximate receptor molecules mediated by, for example, activation of one receptor through a mechanism involving the kinase activity of the other. One particularly relevant example of such a phenomenon is the binding of EGF to the EGFR, resulting in activation of the EGFR kinase domain and cross-phosphorylation of HER2 (Kokai et al., 1989, Cell 58: 287-92; Stern et al., 1988, EMBO J. 7: 995-1001; King et al., 1989, Oncogene 4: 13-18).

3. SUMMARY OF THE INVENTION

HER4 is the fourth member of the EGFR-family of receptor tyrosine kinases and is likely to be involved not only in regulating normal cellular function but also in the loss of normal growth control associated with certain human cancers. In this connection, HER4 appears to be closely connected with certain carcinomas of epithelial origin, such as adenocarcinoma of the breast. As such, its discovery, and the elucidation of the HER4 coding sequence, open a number of novel approaches to the diagnosis and treatment of human cancers in which the aberrant expression and/or function of this cell surface receptor is involved.

The complete nucleotide sequence encoding the prototype HER4 polypeptide of the invention is disclosed herein, and provides the basis for several general aspects of the invention hereinafter described. Thus, the invention includes embodiments directly involving the production and use of HER4 polynucleotide molecules. In addition, the invention provides HER4 polypeptides, such as the prototype HER4 polypeptide disclosed and characterized in the sections which follow. Polypeptides sharing nearly equivalent structural characteristics with the prototype HER4 molecule are also included within the scope of this invention. Furthermore, the invention includes polypeptides which interact with HER4 expressed on the surface of certain cells thereby affecting their growth and/or differentiation. The invention is also directed to anti-HER4 antibodies, which have a variety of uses including but not limited to their use as components of novel

biological approaches to human cancer diagnosis and therapy provided by the invention.

The invention also relates to the discovery of an apparent functional relationship between HER4 and HER2, and the therapeutic aspects of the invention include those which are based on applicants' preliminary understanding of this relationship. Applicants' data strongly suggests that HER4 interacts with HER2 either by heterodimer formation or receptor crosstalk, and that such interaction appears to be one mechanism by which the HER4 receptor mediates effects on cell behavior. The reciprocal consequence is that HER2 activation is in some circumstances mediated through HER4.

4. BRIEF DESCRIPTIONS OF THE FIGURES

FIG. 1. Nucleotide sequence [SEQ ID NO: 1] and deduced amino acid sequence [SEQ ID NO: 2] of HER4 (1308 amino acid residues). Nucleotides are numbered on the left, and amino acids are numbered above the sequence.

FIG. 2. Nucleotide sequence (FIG. 2(A) [SEQ ID NO: 3]; FIG. 2(B) [SEQ ID NO: 5] and deduced amino acid sequence (FIG. 2(A) [SEQ ID NO: 4]; FIG. 2(B) [SEQ ID NO: 6]) of cDNAs encoding HER4 variants. (A) HER4 with alternate 3' end and without autophosphorylation domain. This sequence is identical with that of HER4 shown in FIG. 1 up to nucleotide 3188, where the sequence diverges and the open reading frame stops after 13 amino acids, followed by an extended, unique 3'-untranslated region. (B) HER4 with N-terminal truncation. This sequence contains the 3'-portion of the HER4 sequence where nucleotide position 156 of the truncated sequence aligns with position 2335 of the complete HER4 sequence shown in FIG. 1 (just downstream from the region encoding the ATP-binding site of the HER4 kinase). The first 155 nucleotides of the truncated sequence are unique from HER4 and may represent the 5'-untranslated region of a transcript derived from a cryptic promoter within an intron of the HER4 gene. (Section 6.2.2., *infra*).

FIG. 3. The deduced amino acid sequence of two variant forms of human HER4 aligned with the full length HER4 receptor as represented in FIG. 1. Sequences are displayed using the single-letter code and are numbered on the right with the complete HER4 sequence on top and the variant sequences below. Identical residues are indicated by a colon between the aligned residues. (A) HER4 with alternate 3'-end, lacking an autophosphorylation domain [SEQ ID NO: 4]. This sequence is identical with that of HER4 [SEQ ID NO: 2] shown in FIG. 1 up to amino acid 1045, where the sequence diverges and continues for 13 amino acids before reaching a stop codon. (B) HER4 with N-terminal truncation [SEQ ID NO: 6]. This sequence is identical to the 3'-portion of the HER4 [SEQ ID NO: 2] shown in FIG. 1 beginning at amino acid 768. (Section 6.2.2., *infra*).

FIG. 4. Deduced amino acid sequence of human HER4 [SEQ ID NO: 2] and alignment with other human EGFR-family members (EGFR [SEQ ID NO: 7]; HER2 [SEQ ID NO: 8]; HER3 [SEQ ID NO: 9]) Sequences are displayed using the single-letter code and are numbered on the left. Identical residues are denoted with dots, gaps are introduced for optimal alignment, cysteine residues are marked with an asterisk, and N-linked glycosylation sites are denoted with a plus (+). Potential protein kinase C phosphorylation sites are indicated by arrows (HER4 amino acid positions 679, 685, and 699). The predicted ATP-binding site is shown with 4 circled crosses, C-terminal tyrosines are denoted with open triangles, and tyrosines in HER4 that are conserved with the major autophosphorylation sites in the EGFR are indicated with black triangles. The predicted extracellular domain extends from the boundary of the signal sequence marked by an arrow at position 25, to the hydrophobic transmembrane domain which is overlined from amino acid positions 650 through 675. Various subdomains are labeled on the right: I, II, III, and IV = extracellular subdomains (domains II and IV are cysteine-rich); TM = transmembrane domain; TK = tyrosine kinase domain. Domains I, III, TK are boxed.

FIG. 5. (A) Hydropathy profile of HER4, aligned with (B) Comparison of protein domains for HER4 (1308 amino acids), EGFR (1210 amino acids), HER2 (1255 amino acids), and HER3 (1342 amino acids). The signal peptide is represented by a stippled box, the cysteine-rich extracellular subdomains are hatched, the transmembrane domain is filled, and the cytoplasmic tyrosine kinase domain is stippled. The percent amino acid sequence identities between HER4 and other EGFR-family members are indicated. Sig, signal peptide; I, II, III, and IV, extracellular domains; TM, transmembrane domain; JM, juxtamembrane domain; Caln, calcium influx and internalization domain; 3'UTR, 3' untranslated region.

FIG. 6. Northern blot analysis of mRNA from human tissues hybridized to HER4 probes from (A) the 3'-autophosphorylation domain, and (B) the 5'-extracellular domain (see Section 6.2.3., *infra*). RNA size markers (in kilobases) are shown on the left. Lanes 1 through 8 represent 2 μ g of poly(A)⁺ mRNA from pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart, respectively.

FIG. 7. Immunoblot analysis of recombinant HER4 stably expressed in CHO-K1 cells, according to procedure outlined in Section 7.1.3, *infra*. Membrane preparations from CHO-K1 cells expressing recom-

binant HER4 were separated on 7% SDS-polyacrylamide gels and transferred to nitrocellulose. Blots were hybridized with (A) a monoclonal antibody to the C-terminus of HER2 (Ab3, Oncogene Science, Uniondale, NY) that cross-reacts with HER4 or (B) a sheep anti-peptide polyclonal antibody to a common epitope of HER2 and HER4. Lane 1, parental CHO-K1 cells; lanes 2 - 4, CHO-K1/HER4 cell clones 6, 21, and 3, respectively. Note the 180 kDa HER4 protein and the 130 kDa cross-reactive species. The size in kilodaltons of prestained high molecular weight markers (BioRad, Richmond, CA) is shown on the left.

FIG. 8. Specific activation of HER4 tyrosine kinase by a breast cancer differentiation factor (see Section 8., *infra*). Four recombinant cell lines, each of which was engineered to overexpress a single member of EGFR-family of tyrosine kinase receptors (EGFR, HER2, HER3, and HER4), were prepared according to the methods described in Sections 7.1.2 and 8.1., *infra*. Cells from each of the four recombinant cell lines were stimulated with various ligand preparations and assayed for receptor tyrosine phosphorylation using the assay described in Section 8.2., *infra*. (A) CHO/HER4 #3 cells, (B) CHO/HER2 cells, (C) NRHER5 cells, and (D) 283/HER3 cells. Cells stimulated with: lane 1, buffer control; lane 2, 100 ng/ml EGF; lane 3, 200 ng/ml amphiregulin; lane 4, 10 μ l phenyl column fraction 17 (Section 9, *infra*); lane 5, 10 μ l phenyl column fraction 14 (Section 9., *infra*, and see description of FIG. 9 below). The size (in kilodaltons) of the prestained molecular weight markers are labeled on the left of each panel. The phosphorylated receptor in each series migrates just below the 221 kDa marker. Bands at the bottom of the gels are extraneous and are due to the reaction of secondary antibodies with the antibodies used in the immunoprecipitation.

FIG. 9. Biological and biochemical properties of the MDA-MB-453-cell differentiation activity purified from the conditioned media of HepG2 cells (Section 9., *infra*). (A, B, and C) Induction of morphologic differentiation. Conditioned media from HepG2 cells was subjected to ammonium sulfate fractionation, followed by dialysis against PBS. Dilutions of this material were added to MDA-MB-453 monolayer at the indicated protein concentrations. (A) control; (B) 80 ng per well; (C) 2.0 μ g per well. (D) Phenyl-5PW column elution profile monitored at 230 nm absorbance. (E) Stimulation of MDA-MB-453 tyrosine auto-phosphorylation with the following ligand preparations: None (control with no factor added); TGF- α (50 ng/ml); CM (16-fold concentrated HepG2 conditioned medium tested at 2 μ l and 10 μ l per well); fraction (phenyl column fractions 13 to 20, 10 μ l per well). (F) Densitometry analysis of the phosphorylation signals shown in (E).

FIG. 10. NDF-induced tyrosine phosphorylation of (A) MDA-MB-453 cells (lane 1, mock transfected COS cell supernatant; lane 2, NDF transfected COS cell supernatant); and (B) CHO/HER4 21-2 cells (lanes 1 and 2, mock transfected COS cell supernatant; lanes 3 and 4, NDF transfected COS cell supernatant). See Section 10., *infra*. Tyrosine phosphorylation was determined by the tyrosine kinase stimulation assay described in Section 8.2., *infra*.

FIG. 11. Regional location of the HER4 gene to human chromosome 2 band q33. (A) Distribution of 124 sites of hybridization on human chromosomes. (B) Distribution of autoradiographic grains on diagram of chromosome 2.

FIG. 12. Amino acid sequence of HER4-Ig fusion protein [SEQ ID NO: 10] (Section 5.4., *infra*).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to HER4/p180^{erbB4} ("HER4"), a closely related yet distinct member of the Human EGF Receptor (HER)*neu* subfamily of receptor tyrosine kinases, as well as HER4-encoding polynucleotides (e.g., cDNAs, genomic DNAs, RNAs, anti-sense RNAs, etc.), the production of mature and precursor forms of HER4 from a HER4 polynucleotide coding sequence, recombinant HER4 expression vectors, HER4 analogues and derivatives, anti-HER4 antibodies, HER4 ligands, and diagnostic and therapeutic uses of HER4 polynucleotides, polypeptides, ligands, and antibodies in the field of human oncology and neurobiology.

The invention also reveals an apparent functional relationship between the HER4 and HER2 receptors involving HER4-mediated phosphorylation of HER2, potentially via intracellular receptor crosstalk or receptor dimerization. In this connection, the invention also provides a HER4 ligand capable of inducing cellular differentiation in breast carcinoma cells that appears to involve HER4-mediated phosphorylation of HER2. Furthermore, applicants' data provide evidence that NDF/HRG- α mediate biological effects on certain cells not solely through HER2, as has been reported in the literature, but instead by means of a direct interaction with HER4, or through an interaction with a HER2/HER4 complex. In cell lines expressing both HER2 and HER4, binding of NDF to HER4 may stimulate HER2 either by heterodimer formation of these two related receptors or by intracellular receptor crosstalk.

Unless otherwise indicated, the practice of the present invention utilizes standard techniques of molecular biology and molecular cloning, microbiology, immunology, and recombinant DNA known in the

art. Such techniques are described and explained throughout the literature, and can be found in a number of more comprehensive publications such as, for example, Maniatis et al, *Molecular Cloning: A Laboratory Manual* (Second Edition, 1989).

5 6.1. HER4 POLYNUCLEOTIDES

One aspect of the present invention is directed to HER4 polynucleotides, including recombinant polynucleotides encoding the prototype HER4 polypeptide shown in FIG. 1, polynucleotides which are related or are complementary thereto, and recombinant vectors and cell lines incorporating such recombinant polynucleotides. The term "recombinant polynucleotide" as used herein refers to a polynucleotide of genomic, cDNA, synthetic or semisynthetic origin which, by virtue of its origin or manipulation, is not associated with any portion of the polynucleotide with which it is associated in nature, and may be linked to a polynucleotide other than that to which it is linked in nature, and includes single or double stranded polymers of ribonucleotides, deoxyribonucleotides, nucleotide analogs, or combinations thereof. The term also includes various modifications known in the art, including but not limited to radioactive and chemical labels, methylation, caps, internucleotide modifications such as those with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.) and uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidites, carbamites, etc.), as well as those containing pendant moieties, intercalators, chelators, alkylators, etc. Related polynucleotides are those having a contiguous stretch of about 200 or more nucleotides and sharing at least about 80% homology to a corresponding sequence of nucleotides within the nucleotide sequence disclosed in FIG. 1. Several particular embodiments of such HER4 polynucleotides and vectors are provided in example Sections 6 and 7, *infra*.

HER4 polynucleotides may be obtained using a variety of general techniques known in the art, including molecular cloning and chemical synthetic methods. One method by which the molecular cloning of cDNAs encoding the prototype HER4 polypeptide of the invention (FIG. 1), as well as several HER4 polypeptide variants, is described by way of example in Section 6., *infra*. Conserved regions of the sequences of EGFR, HER2, HER3, and Xmrk are used for selection of the degenerate oligonucleotide primers which are then used to isolate HER4. Since many of these sequences have extended regions of amino acid identity, it is difficult to determine if a short PCR fragment represents a unique molecule or merely the species-specific counterpart of EGFR, HER2, or HER3. Often the species differences for one protein are as great as the differences within species for two distinct proteins. For example, fish Xmrk has regions of 47/55 (85%) amino acid identity to human EGFR, suggesting it might be the fish EGFR, however isolation of another clone that has an amino acid sequence identical to Xmrk in this region (57/57) shows a much higher homology to human EGFR in its flanking sequence (92% amino acid homology) thereby suggesting that it, and not Xmrk, is the fish EGFR (Wittbrodt, J. et al., 1989, *Nature* 342: 415-421). As described in Section 6., *infra*, it was necessary to confirm that a murine HER4/erbB4 PCR fragment was indeed a unique gene, and not the murine homolog of EGFR, HER2, or HER3, by isolating genomic fragments corresponding to murine EGFR, erbB2 and erbB3. Sequence analysis of these clones confirmed that this fragment was a novel member of the EGFR family. Notably a region of the murine clone had a stretch of 60/64 amino acid identity to human HER2, but comparison with the amino acid and DNA sequences of the other EGFR homologs from the same species (mouse) firmly established it encoded a novel transcript.

HER4 polynucleotides may be obtained from a variety of cell sources which produce HER4-like activities and/or which express HER4-encoding mRNA. In this connection, applicants have identified a number of suitable human cell sources for HER4 polynucleotides, including but not limited to brain, cerebellum, pituitary, heart, skeletal muscle, and a variety of breast carcinoma cell lines (see Section 6., *infra*).

For example, polynucleotides encoding HER4 polypeptides may be obtained by cDNA cloning from RNA isolated and purified from such cell sources or by genomic cloning. Either cDNA or genomic libraries of clones may be prepared using techniques well known in the art and may be screened for particular HER4-encoding DNAs with nucleotide probes which are substantially complementary to any portion of the HER4 gene. Various PCR cloning techniques may also be used to obtain the HER4 polynucleotides of the invention. A number of PCR cloning protocols suitable for the isolation of HER4 polynucleotides have been reported in the literature (see, for example, PCR protocols: A Guide to Methods and Applications, Eds. Inis et al., Academic Press, 1990).

For the construction of expression vectors, polynucleotides containing the entire coding region of the desired HER4 may be isolated as full length clones or prepared by splicing two or more polynucleotides together. Alternatively, HER4-encoding DNAs may be synthesized in whole or in part by chemical synthesis

using techniques standard in the art. Due to the inherent degeneracy of nucleotide coding sequences, any polynucleotide encoding the desired HER4 polypeptide may be used for recombinant expression. Thus, for example, the nucleotide sequence encoding the prototype HER4 of the invention provided in FIG. 1 may be altered by substituting nucleotides such that the same HER4 product is obtained.

5 The invention also provides a number of useful applications of the the HER4 polynucleotides of the invention, including but not limited to their use in the preparation of HER4 expression vectors, primers and probes to detect and/or clone HER4, and diagnostic reagents. Diagnostics based upon HER4 polynucleotides include various hybridization and PCR assays known in the art, utilizing HER4 polynucleotides as primers or probes, as appropriate. One particular aspect of the invention relates to a PCR kit comprising
10 a pair of primers capable of priming cDNA synthesis in a PCR reaction, wherein each of the primers is a HER4 polynucleotide of the invention. Such a kit may be useful in the diagnosis of certain human cancers which are characterized by aberrant HER4 expression. For example, certain human carcinomas may overexpress HER4 relative to their normal cell counterparts, such as human carcinomas of the breast. Thus, detection of HER4 overexpression mRNA in breast tissue may be an indication of neoplasia. In another,
15 related embodiment, human carcinomas characterized by overexpression of HER2 and expression or overexpression of HER4 may be diagnosed by a polynucleotide-based assay kit capable of detecting both HER2 and HER4 mRNAs, such a kit comprising, for example, a set of PCR primer pairs derived from divergent sequences in the HER2 and HER4 genes, respectively.

20 6.2. HER4 POLYPEPTIDES

Another aspect of the invention is directed to HER4 polypeptides, including the prototype HER4 polypeptide provided herein, as well as polypeptides derived from or having substantial homology to the amino acid sequence of the prototype HER4 molecule. The term "polypeptide" in this context refers to
25 a polypeptide prepared by synthetic or recombinant means, or which is isolated from natural sources. The term "substantially homologous" in this context refers to polypeptides of about 80 or more amino acids sharing greater than about 80% amino acid homology to a corresponding contiguous amino acid sequence in the prototype HER4 primary structure (FIG. 1). The term "prototype HER4" refers to a polypeptide having the amino acid sequence of precursor or mature HER4 as provided in FIG. 1, which is encoded by
30 the consensus cDNA nucleotide sequence also provided therein, or by any polynucleotide sequence which encodes the same amino acid sequence.

HER4 polypeptides of the invention may contain deletions, additions or substitutions of amino acid residues relative to the sequence of the prototype HER4 depicted in FIG. 1 which result in silent changes thus producing a bioactive product. Such amino acid substitutions may be made on the basis of similarity in
35 polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

40 The HER4 polypeptide depicted in FIG. 1 has all of the fundamental structural features characterizing the EGFR-family of receptor tyrosine kinases (Hanks et al., 1988, Science 241: 42-52). The precursor contains a single hydrophobic stretch of 26 amino acids characteristic of a transmembrane region that bisects the protein into a 625 amino acid extracellular ligand binding domain, and a 633 amino acid C-terminal cytoplasmic domain. The ligand binding domain can be further divided into 4 subdomains (I - IV),
45 including two cysteine-rich regions (II, residues 186-334; and IV, residues 496-633), and two flanking domains (I, residues 29-185; and III, residues 335-495) that may define specificity for ligand binding (Lax et al., 1988, Mol. Cell. Biol. 8:1970-78). The extracellular domain of HER4 is most similar to HER3, where domains II-IV of HER4 share 56-87% identity to the respective domains of HER3. In contrast, the same regions of EGFR and HER2 exhibit 43-51% and 34-46% homology to HER4, respectively (FIG. 4). The 4
50 extracellular subdomains of EGFR and HER2 share 39-50% identity. HER4 also conserves all 50 cysteines present in the extracellular portion of EGFR, HER2, and HER3, except that the HER2 protein lacks the fourth cysteine in domain IV. There are 11 potential N-linked glycosylation sites in HER4, conserving 4 of 12 potential sites in EGFR, 3 of 8 sites in HER2, and 4 of 10 sites in HER3.

Following the transmembrane domain of HER4 is a cytoplasmic juxtamembrane region of 37 amino
55 acids. This region shares the highest degree of homology with EGFR (73% amino acid identity) and contains two consensus protein kinase C phosphorylation sites at amino acid residue numbers 679 (Serine) and 699 (Threonine) in the FIG. 1 sequence, the latter of which is present in EGFR and HER2. Notably, HER4 lacks a site analogous to Thr654 of EGFR. Phosphorylation of this residue in the EGFR appears to

block ligand-induced internalization and plays an important role in its transmembrane signaling (Livneh et al., 1988, Mol. Cell. Biol. 8: 2302-08). HER4 also contains Thr692 analogous to Thr694 of HER2. This threonine is absent in EGFR and HER3 and has been proposed to impart cell-type specificity to the mitogenic and transforming activity of the HER2 kinase (DiFiore et al. 1992, EMBO J. 11: 3827-33). The
 5 extramembrane region of HER4 also contains a MAP kinase consensus phosphorylation site at amino acid number 699 (Threonine), in a position homologous to Thr699 of EGFR which is phosphorylated by MAP kinase in response to EGF stimulation (Takahima et al., 1991, Proc. Natl. Acad. Sci. U.S.A. 88: 2520-25).

The remaining cytoplasmic portion of HER4 consists of a 276 amino acid tyrosine kinase domain, an acidic helical structure of 38 amino acids that is homologous to a domain required for ligand-induced
 10 internalization of the EGFR (Chen et al., 1989, Cell 59:33-43), and a 282 amino acid region containing 18 tyrosine residues characteristic of the autophosphorylation domains of other EGFR-related proteins (FIG. 4). The 276 amino acid tyrosine kinase domain conserves all the diagnostic structural motifs of a tyrosine kinase, and is most related to the catalytic domains of EGFR (79% identity) and HER2 (77% identity), and to a lesser degree, HER3 (63% identity). In this same region, EGFR and HER2 share 83% identity.
 15 Examples of the various conserved structural motifs include the following: the ATP-binding motif (GXGXXG) [SEQ ID NO: 11] with a distal lysine residue that is predicted to be involved in the phosphotransfer reaction (Hanks et al., 198, Science 241: 42-52; Hunter and Cooper, in The Enzymes Vol. 17 (eds. Boyer and Krebs) pp. 191-246 (Academic Press 1988)); tyrosine-kinase specific signature sequences (DLAARN [SEQ ID NO: 12] and PIKWMA [SEQ ID NO: 13]) and Tyr875 (FIG. 4), a residue that frequently serves as an
 20 autophosphorylation site in many tyrosine kinases (Hunter and Cooper, *supra*); and approximately 15 residues that are either highly or completely conserved among all known protein kinases (Plowman et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87: 4905-09; Hanks et al., *supra*). The C-terminal 282 amino acids of HER4 has limited homology with HER2 (27%) and EGFR (19%). However, the C-terminal domain of each EGFR-family receptor is proline-rich and conserves stretches of 2-7 amino acids that are generally centered
 25 around a tyrosine residue. These residues include the major tyrosine autophosphorylation sites of EGFR at Tyr1068, Tyr1086, Tyr1148, and Tyr1173 (FIG. 4, filled triangles; Margolis et al., 1989, J. Biol. Chem. 264: 10667-71).

5.3. RECOMBINANT SYNTHESIS OF HER4 POLYPEPTIDES

30 The HER4 polypeptides of the invention may be produced by the cloning and expression of DNA encoding the desired HER4 polypeptide. Such DNA may be ligated into a number of expression vectors well known in the art and suitable for use in a number of acceptable host organisms, in fused or mature form, and may contain a signal sequence to permit secretion. Both prokaryotic and eukaryotic host
 35 expression systems may be employed in the production of recombinant HER4 polypeptides. For example, the prototype HER4 precursor coding sequence or its functional equivalent may be used in a host cell capable of processing the precursor correctly. Alternatively, the coding sequence for mature HER4 may be used to directly express the mature HER4 molecule. Functional equivalents of the HER4 precursor coding sequence include any DNA sequence which, when expressed inside the appropriate host cell, is capable of
 40 directing the synthesis, processing and/or export of HER4.

Production of a HER4 polypeptide using recombinant DNA technology may be divided into a four-step process for the purposes of description: (1) isolation or generation of DNA encoding the desired HER4 polypeptide; (2) construction of an expression vector capable of directing the synthesis of the desired HER4 polypeptide; (3) transfection or transformation of appropriate host cells capable of replicating and expressing
 45 the HER4 coding sequence and/or processing the initial product to produce the desired HER4 polypeptide; and (4) identification and purification of the desired HER4 product.

5.3.1. ISOLATION OR GENERATION OF HER4 ENCODING DNA

50 HER4-encoding DNA, or functional equivalents thereof, may be used to construct recombinant expression vectors which will direct the expression of the desired HER4 polypeptide product. In a specific embodiment, DNA encoding the prototype HER4 polypeptide (FIG. 1), or fragments or functional equivalents thereof, may be used to generate the recombinant molecules which will direct the expression of the recombinant HER4 product in appropriate host cells. HER4-encoding nucleotide sequences may be
 55 obtained from a variety of cell sources which produce HER4-like activities and/or which express HER4-encoding mRNA. For example, HER4-encoding cDNAs may be obtained from the breast adenocarcinoma cell line MDA-MB-453 (ATCC HTB131) as described in Section 6., *infra*. In addition, a number of human cell sources are suitable for obtaining HER4 cDNAs, including but not limited to various epidermoid and

breast carcinoma cells, and normal heart, kidney, and brain cells (see Section 6.2.3., *infra*).

The HER4 coding sequence may be obtained by molecular cloning from RNA isolated and purified from such cell sources or by genomic cloning. Either cDNA or genomic libraries of clones may be prepared using techniques well known in the art and may be screened for particular HER4-encoding DNAs with nucleotide probes which are substantially complementary to any portion of the HER4 gene. Alternatively, cDNA or genomic DNA may be used as templates for PCR cloning with suitable oligonucleotide primers. Full length clones, i.e., those containing the entire coding region of the desired HER4 may be selected for constructing expression vectors, or overlapping cDNAs can be ligated together to form a complete coding sequence. Alternatively, HER4-encoding DNAs may be synthesized in whole or in part by chemical synthesis using techniques standard in the art.

5.3.2. CONSTRUCTION OF HER4 EXPRESSION VECTORS

Various expression vector/host systems may be utilized equally well by those skilled in the art for the recombinant expression of HER4 polypeptides. Such systems include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the desired HER4 coding sequence; yeast transformed with recombinant yeast expression vectors containing the desired HER4 coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the desired HER4 coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the desired HER4 coding sequence; or animal cell systems infected with recombinant virus expression vectors (e.g., adenovirus, vaccinia virus) including cell lines engineered to contain multiple copies of the HER4 DNA either stably amplified (e.g., CHO/dhfr, CHO/glutamine synthetase) or unstably amplified in double-minute chromosomes (e.g., murine cell lines).

The expression elements of these vectors vary in their strength and specificities. Depending on the host/vector system utilized, any one of a number of suitable transcription and translation elements may be used. For instance, when cloning in mammalian cell systems, promoters isolated from the genome of mammalian cells, (e.g., mouse metallothionein promoter) or from viruses that grow in these cells, (e.g., vaccinia virus 7.5K promoter or Moloney murine sarcoma virus long terminal repeat) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted sequences.

Specific initiation signals are also required for sufficient translation of inserted protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire HER4 gene including its own initiation codon and adjacent sequences are inserted into the appropriate expression vectors, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the HER4 coding sequences to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of transcription attenuation sequences, enhancer elements, etc.

For example, in cases where an adenovirus is used as an expression vector, the desired HER4 coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E3 or E4) will result in a recombinant virus that is viable and capable of expressing HER4 in infected hosts. Similarly, the vaccinia 7.5K promoter may be used. An alternative expression system which could be used to express HER4 is an insect system. In one such system, Autographa californica nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The HER4 coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the HER4 coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat encoded by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. Yet another approach uses retroviral vectors prepared in amphotropic packaging cell lines, which permit high efficiency expression in numerous cells types. This method allows one to assess cell-type specific processing, regulation or function of the inserted protein coding sequence.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences; or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers. (e.g., zinc and cadmium ions for metallothionein promoters). Therefore, expression of the recombinant HER4 polypeptide may be controlled.

This is important if the protein product of the cloned foreign gene is lethal to host cells. Furthermore, modifications (e.g., phosphorylation) and processing (e.g., cleavage) of protein products are important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of protein. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

5.3.3. TRANSFORMANTS EXPRESSING HER4 GENE PRODUCTS

The host cells which contain the recombinant coding sequence and which express the desired HER4 polypeptide product may be identified by at least four general approaches (a) DNA-DNA, DNA-RNA or RNA-antisense RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of HER4 mRNA transcripts in the host cell; and (d) detection of the HER4 product as measured by immunoassay and, ultimately, by its biological activities.

In the first approach, for example, the presence of HER4 coding sequences inserted into expression vectors can be detected by DNA-DNA hybridization using hybridization probes and/or primers for PCR reactions comprising polynucleotides that are homologous to the HER4 coding sequence.

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate (MTX), resistance to methionine sulfoximine (MSX), transformation phenotype, occlusion body formation in baculovirus, (etc.). For example, if the HER4 coding sequence is inserted within a marker gene sequence of the vector, recombinants containing that coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the HER4 sequence under the control of the same or different promoter used to control the expression of the HER4 coding sequence. Expression of the marker in response to induction or selection indicates expression of the HER4 coding sequence. In a particular embodiment described by way of example herein, a HER4 expression vector incorporating glutamine synthetase as a selectable marker is constructed, used to transfect CHO cells, and amplified expression of HER4 in CHO cells is obtained by selection with increasing concentration of MSX.

In the third approach, transcriptional activity for the HER4 coding region can be assessed by hybridization assays. For example, polyadenylated RNA can be isolated and analyzed by Northern blot using a probe homologous to the HER4 coding sequence or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of HER4 can be assessed immunologically, for example by Western blots, immunoassays such as radioimmunoprecipitation, enzyme-linked immunoassays and the like. Alternatively, expression of HER4 may be assessed by detecting a biologically active product. Where the host cell secretes the gene product the cell free media obtained from the cultured transfectant host cell may be assayed for HER4 activity. Where the gene product is not secreted, cell lysates may be assayed for such activity. In either case, assays which measure ligand binding to HER4, HER4 phosphorylation, or other bioactivities of HER4 may be used.

5.4. ANTI-HER4 ANTIBODIES

The invention is also directed to polyclonal and monoclonal antibodies which recognize epitopes of HER4 polypeptides. Anti-HER4 antibodies are expected to have a variety of useful applications in the field of oncology, several of which are described generally below. More detailed and specific descriptions of various uses for anti-HER4 antibodies are provided in the sections and subsections which follow. Briefly, anti-HER4 antibodies may be used for the detection and quantification of HER4 polypeptide expression in cultured cells, tissue samples, and *in vivo*. Such immunological detection of HER4 may be used, for example, to identify, monitor, and assist in the prognosis of neoplasms characterized by aberrant or attenuated HER4 expression and/or function. Additionally, monoclonal antibodies recognizing epitopes from different parts of the HER4 structure may be used to detect and/or distinguish between native HER4 and various subcomponent and/or mutant forms of the molecule. Anti-HER4 antibody preparations are also envisioned as useful biomodulatory agents capable of effectively treating particular human cancers. In addition to the various diagnostic and therapeutic utilities of anti-HER4 antibodies, a number of industrial

and research applications will be obvious to those skilled in the art, including, for example, the use of anti-HER4 antibodies as affinity reagents for the purification of HER4 polypeptides, and as immunological probes for elucidating the biosynthesis, metabolism and biological functions of HER4.

Anti-HER4 antibodies may be useful for influencing cell functions and behaviors which are directly or indirectly mediated by HER4. As an example, modulation of HER4 biological activity with anti-HER4 antibodies may influence HER2 activation and, as a consequence, modulate intracellular signals generated by HER2. In this regard, anti-HER4 antibodies may be useful to effectively block ligand-induced, HER4-mediated activation of HER2, thereby affecting HER2 biological activity. Conversely, anti-HER4 antibodies capable of acting as HER4 ligands may be used to trigger HER4 biological activity and/or initiate a ligand-induced, HER4-mediated effect on HER2 biological activity, resulting in a cellular response such as differentiation, growth inhibition, etc.

Additionally, anti-HER4 antibodies conjugated to cytotoxic compounds may be used to selectively target such compounds to tumor cells expressing HER4, resulting in tumor cell death and reduction or eradication of the tumor. In a particular embodiment, toxin-conjugated antibodies having the capacity to bind to HER4 and internalize into such cells are administered systemically for targeted cytotoxic effect. The preparation and use of radionuclide and toxin conjugated anti-HER4 antibodies are further described in Section 5.5., *infra*.

Overexpression of HER2 is associated with several human cancers. Applicants' data indicate that HER4 is expressed in certain human carcinomas in which HER2 overexpression is present. Therefore, anti-HER4 antibodies may have growth and differentiation regulatory effects on cells which overexpress HER2 in combination with HER4 expression, including but not limited to breast adenocarcinoma cells. Accordingly, this invention includes antibodies capable of binding to the HER4 receptor and modulating HER2 or HER2-HER4 functionality, thereby affecting a response in the target cell. For the treatment of cancers involving HER4-mediated regulation of HER2 biological activity, agents capable of selectively and specifically affecting the intracellular molecular interaction between these two receptors may be conjugated to internalizing anti-HER4 antibodies. The specificity of such agents may result in biological effects only in cells which co-express HER2 and HER4, such as breast cancer cells.

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of HER4. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of anti-HER4 antibodies by immunization with one or more injections of a HER4 polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*.

A monoclonal antibody to an epitope of HER4 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, *Nature* 256, 495-497), and the more recent human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today* 4:72) and EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In addition techniques developed for the production of "chimeric antibodies" by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison et al., 1984, *Proc. Natl. Acad. Sci.*, 81:6851-6855; Neuberger et al., 1984, *Nature*, 312:604-608; Takeda et al., 1985, *Nature*, 314:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce HER4-specific single chain antibodies. Recombinant human or humanized versions of anti-HER4 monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g., Jones et al., 1986, *Nature* 321: 522-25; Reichman et al., 1988, *Nature* 332: 323-27; Verhoeven et al., 1988, *Science* 239: 1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized anti-HER2 monoclonal antibody may also be employed in the production of humanized anti-HER4 antibodies (Carter et al., 1992, *Proc. Natl. Acad. Sci. U.S.A.* 89: 4285-89). Alternatively, techniques for generating a recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant anti-HER4 antibodies (e.g., Huse et al., 1989, *Science* 246: 1275-81).

As an example, anti-HER4 monoclonal antibodies may be generated by immunization of mice with cells selectively overexpressing HER4 (e.g., CHO/HER4 21-2 cells as deposited with the ATCC) or with partially purified recombinant HER4 polypeptides. In one embodiment, the full length HER4 polypeptide (FIG. 1)

may be expressed in Baculovirus systems, and membrane fractions of the recombinant cells used to immunize mice. Hybridomas are then screened on CHO/HER4 cells (e.g., CHO HER4 21-2 cells as deposited with the ATCC) to identify monoclonal antibodies reactive with the extracellular domain of HER4. Such monoclonal antibodies may be evaluated for their ability to block NDF, or HepG2-differentiating factor, binding to HER4; for their ability to bind and stay resident on the cell surface, or to internalize into cells expressing HER4; and for their ability to directly upregulate or downregulate HER4 tyrosine autophosphorylation and/or to directly induce a HER4-mediated signal resulting in modulation of cell growth or differentiation. In this connection, monoclonal antibodies N28 and N29, directed to HER2, specifically bind HER2 with high affinity. However, monoclonal N29 binding results in receptor internalization and down-regulation, morphologic differentiation, and inhibition of HER2 expressing tumor cells in athymic mice. In contrast, monoclonal N28 binding to HER2 expressing cells results in stimulation of autophosphorylation, and an acceleration of tumor cell growth both *in vitro* and *in vivo* (Bacus et al., 1992, Cancer Res. 52: 2580-89; Stancovski et al., 1991, Proc. Natl. Acad. Sci. U.S.A. 88: 8891-85). In yet another embodiment, a soluble recombinant HER4-Immunoglobulin (HER4-Ig) fusion protein is expressed and purified on a Protein A affinity column. The amino acid sequence of one such HER4-Ig fusion protein is provided in FIG. 12. The soluble HER4-Ig fusion protein may then be used to screen phage libraries designed so that all available combinations of a variable domain of the antibody binding site are presented on the surfaces of the phages in the library. Recombinant anti-HER4 antibodies may be propagated from phage which specifically recognize the HER4-Ig fusion protein.

Antibody fragments which contain the idiotype of the molecule may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to HER4 protein.

5.5. DIAGNOSTIC METHODS

The invention also relates to the detection of human neoplastic conditions, particularly carcinomas of epithelial origin, and more particularly human breast carcinomas. In one embodiment, oligomers corresponding to portions of the consensus HER4 cDNA sequence provided in FIG. 1 are used for the quantitative detection of HER4 mRNA levels in a human biological sample, such as blood, serum, or tissue biopsy samples, using a suitable hybridization or PCR format assay, in order to detect cells or tissues expressing abnormally high levels of HER4 as an indication of neoplasia. In a related embodiment, detection of HER4 mRNA may be combined with the detection HER2 mRNA overexpression, using appropriate HER2 sequences, to identify neoplasias in which a functional relationship between HER2 and HER4 may exist.

In another embodiment, labeled anti-HER4 antibodies or antibody derivatives are used to detect the presence of HER4 in biological samples, using a variety of immunoassay formats well known in the art, and may be used for in situ diagnostic radioimmunoimaging. Current diagnostic and staging techniques do not routinely provide a comprehensive scan of the body for metastatic tumors. Accordingly, anti-HER4 antibodies labeled with, for example, fluorescent, chemiluminescent, and radioactive molecules may overcome this limitation. In a preferred embodiment, a gamma-emitting diagnostic radionuclide is attached to a monoclonal antibody which is specific for an epitope of HER4, but not significantly cross-reactive with other EGFR-family members. The labeled antibody is then injected into a patient systemically, and total body imaging for the distribution and density of HER4 molecules is performed using gamma cameras, followed by localized imaging using computerized tomography or magnetic resonance imaging to confirm and/or evaluate the condition, if necessary. Preferred diagnostic radionuclides include but are not limited to technetium-99m, indium-111, iodine-123, and iodine-131.

Recombinant antibody-metallothionein chimeras (Ab-MTs) may be generated as recently described (Das et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89: 9749-53). Such Ab-MTs can be loaded with technetium-99m by virtue of the metallothionein chelating function, and may offer advantages over chemically conjugated chelators. In particular, the highly conserved metallothionein structure may result in minimal immunogenicity.

5.6. TARGETED CANCER THERAPY

The invention is also directed to methods for the treatment of human cancers involving abnormal expression and/or function of HER4 and cancers in which HER2 overexpression is combined with the proximate expression of HER4, including but not limited to human breast carcinomas and other neoplasms overexpressing HER4 or overexpressing HER2 in combination with expression of HER4. The cancer therapy methods of the invention are generally based on treatments with unconjugated, toxin- or radionuclide-conjugated HER4 antibodies, ligands, and derivatives or fragments thereof. In one specific embodiment, such HER4 antibodies may be used for systemic and targeted therapy of certain cancers overexpressing HER2 and/or HER4, such as metastatic breast cancer, with minimal toxicity to normal tissues and organs. Importantly, in this connection, an anti-HER2 monoclonal antibody has been shown to inhibit the growth of human tumor cells overexpressing HER2 (Bacus et al., 1992, Cancer Res. 52: 2580-89). In addition to conjugated antibody therapy, modulation of NDF signaling through HER4 may provide a means to affect the growth and differentiation of cells overexpressing HER2, such as certain breast cancer cells, using HER4-neutralizing monoclonal antibodies, NDF/HER4 antagonists, monoclonal antibodies or ligands which act as super-agonists for HER4 activation, or agents which block the interaction between HER2 and HER4, either by disrupting heterodimer formation or by blocking HER-mediated phosphorylation of the HER2 substrate.

For targeted immunotoxin-mediated cancer therapy, various drugs or toxins may be conjugated to anti-HER4 antibodies and fragments thereof, such as plant and bacterial toxins. For example, ricin, a cytotoxin from the *Ricinus communis* plant may be conjugated to an anti-HER4 antibody using methods known in the art (e.g., Blakey et al., 1988, Prog. Allergy 45: 50-80; Marsh and Neville, 1988, J. Immunol. 140: 3674-78). Once ricin is inside the cell cytoplasm, its A chain inhibits protein synthesis by inactivating the 60S ribosomal subunit (May et al., 1989, EMBO J. 8: 301-08). Immunotoxins of ricin are therefore extremely cytotoxic. However, ricin immunotoxins are not ideally specific because the B chain can bind to virtually all cell surface receptors, and immunotoxins made with ricin A chain alone have increased specificity. Recombinant or deglycosylated forms of the ricin A chain may result in improved survival (i.e., slower clearance from circulation) of the immunotoxins. Methods for conjugating ricin A chain to antibodies are known (e.g., Vitella and Thorpe, in: Seminars in Cell Biology, pp47-58; Saunders, Philadelphia 1991). Additional toxins which may be used in the formulation of immunotoxins include but are not limited to daunorubicin, methotrexate, ribosome inhibitors (e.g., trichosanthin, trichokirin, geldonin, saporin, mormordin, and pokeweed antiviral protein) and various bacterial toxins (e.g., *Pseudomonas* endotoxin). Immunotoxins for targeted cancer therapy may be administered by any route which will result in antibody interaction with the target cancer cells, including systemic administration and injection directly to the site of tumor.

For targeted radiotherapy using anti-HER4 antibodies, preferred radionuclides for labeling include alpha, beta, and Auger electron emitters. Examples of alpha emitters include astatine 211 and bismuth 212; beta emitters include iodine 131, rhenium 188, copper 67 and yttrium 90; and iodine 125 is an example of an Auger electron emitter.

5.7. ASSAYS FOR THE IDENTIFICATION OF HER4 LIGANDS

Cell lines overexpressing a single member of the EGFR-family can be generated by transfection of a variety of parental cell types with an appropriate expression vector as described in section 7., *infra*. Candidate ligands, or partially purified preparations, may be applied to such cells and assayed for receptor binding and/or activation. For example, a CHO-K1 cell line transfected with a HER4 expression plasmid and lacking detectable EGFR, HER2, or HER3 may be used to screen for HER4-specific ligands. A particular embodiment of such a cell line is described in Section 7., *infra* and has been deposited with the ATCC (CHO/HER4 21-2). Ligands may be identified by detection of HER4 autophosphorylation, stimulation of DNA synthesis, induction of morphologic differentiation, relief from serum or growth factor requirements in the culture media, and direct binding of labeled purified growth factor. The invention also relates to a bioassay for testing potential analogs of HER4 ligands based on a capacity to affect a biological activity mediated by the HER4 receptor.

5.8 HER4 ANALOGUES

The production and use of derivatives, analogues and peptides related to HER4 are also envisioned and are within the scope of the invention. Such derivatives, analogues and peptides may be used to compete with native HER4 for binding of HER4 specific ligand, thereby inhibiting HER4 signal transduction and function. The inhibition of HER4 function may be utilized in several applications, including but not limited to

the treatment of cancers in which HER4 biological activity is involved.

In a specific embodiment, a series of deletion mutants in the HER4 nucleotide coding sequence depicted in FIG.1 may be constructed and analyzed to determine the minimum amino acid sequence requirements for binding of a HER4 ligand. Deletion mutants of the HER4 coding sequence may be constructed using methods known in the art which include but are not limited to use of nucleases and/or restriction enzymes; site-directed mutagenesis techniques, PCR, etc. The mutated polypeptides expressed may be assayed for their ability to bind HER4 ligand.

The DNA sequence encoding the desired HER4 analogue may then be cloned into an appropriate expression vector for overexpression in either bacteria or eukaryotic cells. Peptides may be purified from cell extracts in a number of ways including but not limited to ion-exchange chromatography or affinity chromatography using HER4 ligand or antibody. Alternatively, polypeptides may be synthesized by solid phase techniques followed by cleavage from resin and purification by high performance liquid chromatography.

6. EXAMPLE: ISOLATION OF cDNAs ENCODING HER4

EGFR and the related proteins, HER2, HER3, and Xmrk exhibit extensive amino acid homology in their tyrosine kinase domains (Kaplan et al., 1991, *Nature* 350: 158-160; Wen et al., 1992, *Cell* 69: 559-72; Holmes et al., 1992, *Science* 256: 1205-10; Hirai et al., 1987, *Science* 238: 1717-20). In addition, there is strict conservation of the exon-intron boundaries within the genomic regions that encode these catalytic domains (Wen et al., *supra*; Lindberg and Hunter, 1990, *Mol. Cell. Biol.* 10: 6316-24; and unpublished observations). Degenerate oligonucleotide primers were designed based on conserved amino acids encoded by a single exon or adjacent exons from the kinase domains of these four proteins. These primers were used in a polymerase chain reaction (PCR) to isolate genomic fragments corresponding to murine EGFR, erbB2 and erbB3. In addition, a highly related DNA fragment (designated MER4) was identified as distinct from these other genes. A similar strategy was used to obtain a cDNA clone corresponding to the human homologue of MER4 from the breast cancer cell line, MDA-MB-453. Using this fragment as a probe, several breast cancer cell lines and human heart were found to be an abundant source of the EGFR-related transcript. cDNA libraries were constructed using RNA from human heart and MDA-MB-453 cells, and overlapping clones were isolated spanning the complete open reading frame of HER4/erbB4.

6.1. MATERIALS AND METHODS

6.1.1. MOLECULAR CLONING

Several pools of degenerate oligonucleotides were synthesized based on conserved sequences from EGFR-family members (Table I).

5'-ACNGTNTGGGARYTNAYHAC-3' [SEQ ID NO: 14]; 5'-CAYGTNAARATHACNGAYTTYGG-3' [SEQ ID NO: 15]; 5'-GACGAATTCNATHAARTGGATGGC [SEQ ID NO: 16]; 5'-ACAYTTNARDATDATCATRTANAC-3' [SEQ ID NO: 17]; 5'-AANGTCATNARYTCCCA-3' [SEQ ID NO: 18]; 5'-TCCAGNGCGATCCAYTT-DATNGG-3' [SEQ ID NO: 19]; 5'-GGRTCDATCATCCARCCT-3' [SEQ ID NO: 20]; 5'-CTGCTGTCCAGCATC-GATCAT-3' [SEQ ID NO: 21]; TVWELMT [SEQ ID NO: 22]; HVKITDFG [SEQ ID NO: 23]; PIKWMA [SEQ ID NO: 13]; VYMIILK [SEQ ID NO: 24]; WELMTF [SEQ ID NO: 25]; PIKWMALE [SEQ ID NO: 26]; CWMIDP [SEQ ID NO: 27]

Total genomic DNA was isolated from subconfluent murine K1735 melanoma cells and used as a template with these oligonucleotide primers in a 40 cycle PCR amplification. PCR products were resolved on agarose gels and hybridized to ³²P-labeled probes from the kinase domain of human EGFR and HER2. Distinct DNA bands were isolated and subcloned for sequence analysis. Using the degenerate oligonucleotides H4VWELM and H4VYMIIL as primers in a PCR amplification (Plowman et al., 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87: 4905-09), one clone (MER4-85) was identified that contained a 144 nucleotide insert corresponding to murine erbB4. This ³²P-labeled insert was used to isolate a 17-kilobase fragment from a murine T-cell genomic library (Stratagene, La Jolla, CA) that was found to contain two exons of the murine erbB4 gene. A specific oligonucleotide (4M3070) was synthesized based on the DNA sequence of an erbB4 exon, and used in a PCR protocol with a degenerate 5'-oligonucleotide (H4PIKWMA) on a template of single stranded MDA-MB-453 cDNA. This reaction generated a 260 nucleotide fragment (pMDAPIK) corresponding to human HER4. cDNA libraries were constructed in lambda ZAP II (Stratagene) from oligo(dT)- and specific-primed MDA-MB453 and human heart RNA (Plowman et al., *supra*; Plowman et al., 1990, *Mol. Cell. Biol.* 10: 1969-81). HER4-specific clones were isolated by probing the libraries with the

³²P-labeled insert from pMDA-PIK. To complete the cloning of the 5'-portion of HER4, we used a PCR strategy to allow for rapid amplification of cDNA ends (Plowman et al., *supra*; Frohman et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85: 8998-9002). All cDNA clones and several PCR generated clones were sequenced on both strands using T7 polymerase with oligonucleotide primers (Tabor and Richardson, 1987, Proc. Natl. Acad. Sci. U.S.A. 84: 4767-71).

TABLE I
OLIGONUCLEOTIDE PREPARATIONS FOR CLONING HER4

Designation	Nucleotide Sequence ¹	Degeneracy	Encoded Sequence	Orientation
H4TVWELM	5'-ACNGTNTGGGARYTNAYHAC-3'	256-fold	TVWELMT	sense
H4KITDFG	5'-CAYGTNAARATHACNGAYTTYGG-3'	768-fold	HVKITDFG	sense
H4PIKMA	5'-GACGAATTCNATHAARTGGATGGC	48-fold	PIKMA	sense
H4VYMIL	5'-ACAYTTNARDATDATCATRTANAC-3'	576-fold	VYMILK	antisense
H4WELMTF	5'-AANGTCATHARYTCCCA-3'	32-fold	WELMTF	antisense
H4PIKMA	5'-TCCAGNCGGATCCAYTTDATNGG-3'	96-fold	PIKMALE	antisense
H4CMIDP	5'-GGRTCDATCATCCARCT-3'	12-fold	CMIDP	antisense
4H3070	5'-CTGCTGTCAGCATCGATCAT-3'	zero	erbB4 exon	antisense

¹Degenerate nucleotide residue designations:

D = A, G, or T;
H = A, C, or T;
N = A, C, G, or T;
R = A or G; and
Y = C or T.

6.1.2. NORTHERN BLOT ANALYSIS

3'- and 5'-HER4 specific [α -³²P]UTP-labeled antisense RNA probes were synthesized from the linearized plasmids pHt1B1.6 (containing an 800 bp HER4 fragment beginning at nucleotide 3098) and p5'H4E7 (containing a 1 kb fragment from the 5'-end of the HER4 sequence), respectively. For tissue distribution analysis (Section 6.2.2., *infra*), the Northern blot (Clontech, Palo Alto, CA) contained 2 μ g poly(A)⁺ mRNA per lane from 8 human tissue samples immobilized on a nylon membrane. The filter was prehybridized at 60°C for several hours in RNA hybridization mixture (50% formamide, 5XSSC, 0.5% SDS, 10X Denhardt's solution, 100 μ g/ml denatured herring sperm DNA, 100 μ g/ml tRNA, and 10 μ g/ml polyadenosine) and hybridized in the same buffer at 60°C, overnight with 1-1.5 \times 10⁶ cpm/ml of ³²P-labeled antisense RNA probe. The filters were washed in 0.1XSSC/0.1% SDS, 65°C, and exposed overnight on a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

6.1.3. SEMI-QUANTITATIVE PCR DETECTION OF HER4

RNA was isolated from a variety of human cell lines, fresh frozen tissues, and primary tumors. Single stranded cDNA was synthesized from 10 μ g of each RNA by priming with an oligonucleotide containing a T₁₇ track on its 3'-end (XSCT17:5'GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3') [SEQ ID NO: 28]. 1% or 5% of each single strand template preparation was then used in a 35 cycle PCR reaction with two HER4-specific oligonucleotides: 4H2674: 5'-GAAGAAAGACGACTCGTTCATCGG-3' [SEQ ID NO: 29], and 4H2985: 5'-GACCATGACCATGTAAACGTCAATA-3' [SEQ ID NO: 30]. Reaction products were electrophoresed on 2% agarose gels, stained with ethidium bromide and photographed on a UV light box. The relative intensity of the 291-bp HER4-specific bands were estimated for each sample as shown in Table II.

6.2.1. SEQUENCE ANALYSIS OF cDNA CLONES ENCODING HER4

cDNA clones encoding parts of the HER4 coding and non-coding nucleotide sequences were isolated by PCR cloning according to the method outlined in Section 6.1.1., *supra*. The complete HER4 nucleotide sequence assembled from these cDNAs is shown in FIG. 1 and contains a single open reading frame encoding a polypeptide of 1308 amino acids. The HER4 coding region is flanked by a 33 nucleotide 5'-untranslated region and a 1517 nucleotide 3'-untranslated region ending with a poly(A) tail. A 25 amino acid hydrophobic signal sequence follows a consensus initiating methionine at position number 1 in the amino acid sequence depicted in FIG. 1. In relation to this signal sequence, the mature HER4 polypeptide would be predicted to begin at amino acid residue number 26 in the sequence depicted in FIG. 1 (Gln), followed by the next 1283 amino acids in the sequence. Thus the prototype mature HER4 of the invention is a polypeptide of 1284 amino acids, having a calculated Mr of 144,260 daltons and an amino acid sequence corresponding to residues 26 through 1308 in FIG. 1.

Comparison of the HER4 nucleotide and deduced amino acid sequences (FIG. 1) with the available DNA and protein sequence databases indicated that the HER4 nucleotide sequence is unique, and revealed a 60/64 amino acid identity with HER2 and a 54/54 amino acid identity to a fragment of a rat EGFR homolog, tyro-2.

6.2.2. SEQUENCE ANALYSIS OF RELATED cDNAs

Several cDNAs encoding polypeptides related to the prototype HER4 polypeptide (FIG. 1) were also isolated from the MDA-MB-453 cDNA library and comprised two forms.

The first alternative type of cDNA was identical to the consensus HER4 nucleotide sequence up to nucleotide 3168 (encoding Arg at amino acid position 1045 in the FIG. 1 sequence) and then abruptly diverges into an apparently unrelated sequence (FIG. 2A, FIG. 3A). Downstream from this residue the open reading frame continues for another 13 amino acids before reaching a stop codon followed by a 2 kb 3'-untranslated sequence and poly(A) tail. This cDNA would be predicted to result in a HER4 variant having the C-terminal autophosphorylation domain of the prototype HER4 deleted.

A second type of cDNA was isolated as 4 independent clones each with a 3'-sequence identical to the HER4 consensus, but then diverging on the 5'-side of nucleotide 2335 (encoding Glu at amino acid position 768 in the FIG. 1 sequence), continuing upstream for only another 114-154 nucleotides (FIG. 2B, FIG. 3B). Nucleotide 2335 is the precise location of an intron-exon junction in the HER2 gene (Coussens et al., 1985, Science 230: 1132-39; Semba et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82: 6497-6501), suggesting these cDNAs could be derived from mRNAs that have initiated from a cryptic promoter within the flanking intron. These 5'-truncated transcripts contain an open reading frame identical to that of the HER4 cDNA sequence of FIG. 1, beginning with the codon for Met at amino acid position 772 in FIG. 1. These cDNAs would be predicted to encode a cytoplasmic HER4 variant polypeptide that initiates just downstream from the ATP-binding domain of the HER4 kinase.

6.2.3. HUMAN TISSUE DISTRIBUTION OF HER4 EXPRESSION

Northern blots of poly(A)+ mRNA from human tissue samples were hybridized with antisense RNA probes to the 3'-end of HER4, encoding the autophosphorylation domain, as described in Section 6.1.2., *supra*. A HER4 mRNA transcript of approximately 6kb was identified, and was found to be most abundant in the heart and skeletal muscle (FIG. 6A). An mRNA of greater than approximately 15 kb was detected in the brain, with lower levels also detected in heart, skeletal muscle, kidney, and pancreas tissue samples.

The same blot was stripped and rehybridized with a probe from the 5'-end of HER4, within the extracellular domain coding region, using identical procedures. This hybridization confirmed the distribution of the 15 kb HER4 mRNA species, and detected a 6.5 kb mRNA species in heart, skeletal muscle, kidney, and pancreas tissue samples (FIG. 6B) with weaker signals in lung, liver, and placenta. In addition, minor transcripts of 1.7-2.6 kb were also detected in pancreas, lung, brain, and skeletal muscle tissue samples. The significance of the different sized RNA transcripts is not known.

Various human tissues were also examined for the presence of HER4 mRNA using the semi-quantitative PCR assay described in Section 6.1.3., *supra*. The results are shown in Table II, together with results of the assay on primary tumor samples and neoplastic cell lines (Section 6.2.4., immediately below). These results correlate well with the Northern and solution hybridization analysis results on the selected RNA samples. The highest levels of HER4 transcript expression were found in heart, kidney, and brain tissue samples. In addition, high levels of HER4 mRNA expression were found in parathyroid, cerebellum,

pituitary, spleen, testis, and breast tissue samples. Lower expression levels were found in thymus, lung, salivary gland, and pancreas tissue samples. Finally, low or negative expression was observed in liver, prostate, ovary, adrenal, colon, duodenum, epidermis, and bone marrow samples.

5 **6.2.4. HER4 mRNA EXPRESSION IN PRIMARY TUMORS AND VARIOUS CELL LINES OF NEOPLASTIC ORIGIN**

HER4 mRNA expression profiles in several primary tumors and a number of cell lines of diverse neoplastic origin were determined with the semi-quantitative PCR assay (Section 6.1.3, *supra*) using
 10 primers from sequences in the HER4 kinase domain. The results are included in Table II. This analysis detected the highest expression of HER4 RNA in 4 human mammary adenocarcinoma cell lines (T-47D, MDA-MB-453, BT-474, and H3398), and in neuroblastoma (SK-N-MC), and pancreatic carcinoma (Hs766T) cell lines. Intermediate expression was detected in 3 additional mammary carcinoma cell lines (MCF-7, MDA-MB-330, MDA-MB-381). Low or undetectable expression was found in other cell lines derived from
 15 carcinomas of the breast (MDA-MB-231, MDA-MB-157, MDA-MB-468, SK-BR-3), kidney (Caki-1, Caki-2, G-401), liver (SK-HEP-1, HepG2), pancreas (PANC-1, AsPC-1, Capan-1), colon (HT-29), cervix (CaSki), vulva (A-41), ovary (PA-1, Caov-3), melanoma (SK-MEL-28), or in a variety of leukemic cell lines. Finally, high level expression was observed in Wilms (kidney) and breast carcinoma primary tumor samples.

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TABLE II
HER4 EXPRESSION BY PRC ANALYSIS

	VERY STRONG	STRONG	MEDIUM
6	T47D (breast)	MDA-MB-453 (breast)	MCF-7 (breast)
		BT-474 (breast)	MDA-MB-330 (breast)
		H3396 (breast)	MDA-MB-157 (breast)
		Hs766T (pancreatic)	JEG-3 (choriocarcinoma)
10		SK-N-MC (neural)	HEPM (palate)
		Wilms Tumor (kidney)	458 (medullablastoma)
			Breast Carcinoma
	Kidney	Brain	Skeletal Muscle
	Heart	Cerebellum	Thymus
15	Parathyroid	Pituitary	Pancreas
		Breast	Lung
		Testis	Salivary Gland
		Spleen	
20		WEAK	NEGATIVE
		MDA-MB-231 (breast)	MDA-MB-468 (breast)
		MDA-MB-157 (breast)	G-401 (kidney)
		SK-BR-3 (breast)	HepG2 (liver)
		A-431 (vulva)	PANC-1 (pancreas)
25		Caki-1 (kidney)	AsPC-1 (pancreas)
		Caki-2 (kidney)	Capan-1 (pancreas)
		SK-HEP-1 (liver)	HT-29 (colon)
		THP-1 (macrophage)	CaSki (cervix)
			PA-1 (ovary)
	Prostate		Caov-3 (ovary)
30	Adrenal		SK-MEL-28 (melanoma)
	Ovary		HUF (fibroblast)
	Colon		H2981 (lung)
	Placenta		Ovarian tumor
35			GEO (colon)
			ALL bone marrow
			AML bone marrow
			Duodenum
			Epidermis
			Liver
40			Bone marrow stroma

7. EXAMPLE: RECOMBINANT EXPRESSION OF HER4

7.1. MATERIALS AND METHODS

7.1.1. CHO-K1 CELLS AND CULTURE CONDITIONS

CHO-K1 cells were obtained from the ATCC (Accession Number CCL 81). These cells lack any detectable EGFR, HER2, or HER3 by immunoblot, tyrosine phosphorylation, and ³⁵S-labeled immunoprecipitation analysis. Transfected cell colonies expressing HER4 were selected in glutamine-free Glasgow modified Eagle's medium (GMEM-S, Gibco) supplemented with 10% dialyzed fetal bovine serum an increasing concentrations of methionine sulfoximine (Bebbington, 1991, in Methods: A Companion to Methods in Enzymology 2: 136-145 Academic Press).

7.1.2. EXPRESSION VECTOR CONSTRUCTION AND TRANSFECTIONS

The complete 4 kilobase coding sequence of prototype HER4 was reconstructed and inserted into a glutamine synthetase expression vector, pEE14, under the control of the cytomegalovirus immediate-early promoter (Bebbington, *supra*) to generate the HER4 expression vector pEEHER4. This construct (pEEHER4) was linearized with MluI and transfected into CHO-K1 cells by calcium phosphate precipitation using standard techniques. Cells were placed on selective media consisting of GMEM-S supplemented with 10% dialyzed fetal bovine serum and methionine sulfoximine at an initial concentration of 25 μ M (L-MSX) as described in Bebbington, *supra*, for the selection of initial resistant colonies. After 2 weeks, isolated colonies were transferred to 48-well plates and expanded for HER4 expression immunoassays as described immediately below. Subsequent rounds of selection using higher concentrations of MSX were used to isolate cell colonies tolerating the highest concentrations of MSX. A number of CHO/HER4 clones selected at various concentrations of MSX were isolated in this manner.

7.1.3. HER4 EXPRESSION IMMUNOASSAY

Confluent cell monolayers were scraped into hypotonic lysis buffer (10 mM Tris pH7.4, 1 mM KCl, 2 mM MgCl₂) at 4°C, dounce homogenized with 30 strokes, and the cell debris was removed by centrifugation at 3500 x g, 5 min. Membrane fractions were collected by centrifugation at 100,000 x g, 20 min, and the pellet was resuspended in hot Laemmli sample buffer with 2-mercaptoethanol. Expression of the HER4 polypeptide was detected by immunoblot analysis on solubilized cells or membrane preparations using HER2 immunoreagents generated to either a 19 amino acid region of the HER2 kinase domain, which coincidentally is identical to the HER4 sequence (residues 927-945), or to the C-terminal 14 residues of HER2, which share a stretch of 7 consecutive residues with a region near the C-terminus of HER4. On further amplification, HER4 was detected from solubilized cell extracts by immunoblot analysis with PY20 anti-phosphotyrosine antibody (ICN Biochemicals), presumably reflecting autoactivation and auto-phosphorylation of HER4 due to receptor aggregation resulting from aberrantly high receptor density. More specifically, expression was detected by immunoblotting with a primary murine monoclonal antibody to HER2 (Neu-Ab3, Oncogene Science) diluted 1:50 in blotto (2.5% dry milk, 0.2% NP40 in PBS) using ¹²⁵I-goat anti-mouse Ig F(ab')₂ (Amersham, UK) diluted 1:500 in blotto as a second antibody. Alternatively, a sheep polyclonal antipeptide antibody against HER2 residues 929-947 (Cambridge Research Biochemicals, Valleystream, NY) was used as a primary immunoreagent diluted 1:100 in blotto with ¹²⁵I-Protein G (Amersham) diluted 1:200 in blotto as a second antibody. Filters were washed with blotto and exposed overnight on a phosphorimager (Molecular Dynamics).

7.2. RESULTS

CHO-K1 cells transfected with a vector encoding the complete human prototype HER4 polypeptide were selected for amplified expression in media containing increasing concentrations of methionine sulfoximine as outlined in Section 7.1., et seq., *supra*. Expression of HER4 was evaluated using the immunoassay described in Section 7.1.3., *supra*. Several transfected CHO-K1 cell clones stably expressing HER4 were isolated. One particular clone, CHO/HER4 21-2, was selected in media supplemented with 250 μ M MSX, and expresses high levels of HER4. CHO/HER4 21-2 cells have been deposited with the ATCC.

Recombinant HER4 expressed in CHO/HER4 cells migrated with an apparent Mr of 180,000, slightly less than HER2, whereas the parental CHO cells showed no cross-reactive bands (FIG. 7A). In addition, a 130 kDa band was also detected in the CHO/HER4 cells, and presumably represents a degradation product of the 180 kDa mature protein. CHO/HER4 cells were used to identify ligand specific binding and autophosphorylation of the HER4 tyrosine kinase (see Section 9., et seq., *infra*).

8. EXAMPLE: ASSAY FOR DETECTING EGFR-FAMILY LIGANDS

8.1. CELL LINES

A panel of four recombinant cell lines, each expressing a single member of the human EGFR-family, were generated for use in the tyrosine kinase stimulatory assay described in Section 8.2., below. The cell line CHO/HER4 3 was generated as described in Section 7.1.2, *supra*.

CHO/HER2 cells (clone 1-2500) were selected to express high levels of recombinant human p185^{erbB2} by dihydrofolate reductase-induced gene amplification in dhfr-deficient CHO cells. The HER2 expression

plasmid, cDNeu, was generated by insertion of a full length HER2 coding sequence into a modified pCDM8 (Invitrogen, San Diego, CA) expression vector (Seed and Aruff, 1987, Proc. Natl. Acad. Sci. U.S.A. 84: 3365-69) in which an expression cassette from pSV2DHFR (containing the murine dhfr cDNA driven by the SV40 early promoter) has been inserted at the pCDM8 vector's unique BamHI site. This construct drives

6 HER2 expression from the CMV immediate-early promoter.

NRHER5 cells (Velu et al., 1987, Science 1408-10) were obtained from Dr. Hsing-Jien Kung (Case Western Reserve University, Cleveland, OH). This murine cell line was clonally isolated from NR6 cells infected with a retrovirus stock carrying the human EGFR, and was found to have approximately 10^6 human EGFRs per cell.

10 The cell line 293/HER3 was selected for high level expression of p160^{erbB3}. The parental cell line, 293 human embryonic kidney cells, constitutively expresses adenovirus E1a and have low levels of EGFR expression. This line was established by cotransfection of linearized cHER3 (Plowman et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87: 4905-09) and pMC1neoPolyA (neomycin selectable marker with an Herpes simplex thymidine kinase promoter, Stratagene), with selection in DMEM/F12 media containing 500 µg/ml

15 G418.

8.2. TYROSINE KINASE STIMULATION ASSAY

Cells were plated in 6-well tissue culture plates (Falcon), and allowed to attach at 37°C for 18-24 hr. Prior to the assay, the cells were changed to serum-free media for at least 1 hour. Cell monolayers were then incubated with the amounts of ligand preparations indicated in Section 7.3., below for 5 min at 37°C. Cells were then washed with PBS and solubilized on ice with 0.5 ml PBSTDS containing phosphatase inhibitors (10 mM NaHPO₄, 7.25, 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.2% sodium azide, 1 mM NaF, 1 mM EGTA, 4 mM sodium orthovanadate, 1% aprotinin, 5 µg/ml leupeptin). Cell debris was removed by centrifugation (12000 x g, 15 min, 4°C) and the cleared supernatant reacted with 1 µg murine monoclonal antibody to phosphotyrosine (PY20, ICN Biochemicals, Cleveland, Ohio) for CHO/HER4 and 293/HER3 cells, or 1 µg murine monoclonal antibody to HER2 (Neu-Ab3, Oncogene Sciences) for CHO/HER2 cells, or 1 µg murine monoclonal antibody EGFR-1 to human EGFR (Amersham) for NRHER5 cells. Following a 1 hr incubation at 4°C, 30 µl of a 1:1 slurry (in PBSTDS) of anti-mouse IgG-agarose (for PY20 and Neu-Ab3 antibodies) or protein A-sepharose (for EGFR-R1 antibody) was added and the incubation was allowed to continue an additional 30 minutes. The beads were washed 3 times in PBSTDS and the complexes resolved by electrophoresis on reducing 7% SDS-polyacrylamide gels. The gels were transferred to nitrocellulose and blocked in TNET (10 mM Tris pH7.4, 75 mM NaCl, 0.1% Tween-20, 1 mM EDTA). PY20 antiphosphotyrosine antibody diluted 1:1000 in TNET was used as the primary

35 antibody followed by ¹²⁵I-goat anti-mouse Ig F(ab')₂ diluted 1:500 in TNET. Blots were washed with TNET and exposed on a phosphorimager (Molecular Dynamics).

8.3. RESULTS

40 Several EGF-family member polypeptide and ligand preparations were tested for their ability to stimulate tyrosine phosphorylation of each of four EGFR-family receptors expressed in recombinant CHO cells using the tyrosine phosphorylation stimulation assay described in Section 8.2., above. The particular preparations tested for each of the four recombinant cell lines and the results obtained in the assay are tabulated below, and autoradiographs of some of these results are shown in FIG. 8.

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TABLE III

PREPARATION	STIMULATION OF TYR PHOSPHORYLATION OF EGFR-FAMILY RECEPTORS			
	RECOMBINANT CELLS			
	CHO/HER4#3	CHO/HER2	NRHER5	293/HER3
EGF	-	-	+	-
AMPHIREGULIN	-	-	+	-
TGF- α	-	-	+	-
HB-EGF	-	-	+	-
FRACTION 17*	+	-	-	-
FRACTION 14*	-	-	-	-

* The identification of the HER4 tyrosine kinase stimulatory activity within the conditioned media of HepG2 cells and the isolation of these preparations is described in Section 9, *infra*.

The results indicate that EGF, AR, TGF- α , and HB-EGF, four related ligands which mediate their growth regulatory signals in part through interaction with EGFR, were able to stimulate tyrosine phosphorylation of EGFR expressed in recombinant NIH3T3 cells (for EGF, see FIG. 8C, lane 2), but not HER4, HER2, or HER3 expressed in recombinant CHO or 293 cells (FIG. 8A, B, D, lanes 2 and 3). Additionally, as discussed in more detail below, the assay identified a HepG2-derived preparation (fraction 17) as a HER4 ligand capable of specifically stimulating tyrosine phosphorylation of HER4 expressed in CHO/HER4 cells alone.

9. EXAMPLE: ISOLATION OF A HER4 LIGAND

9.1. MATERIALS AND METHODS

9.1.1. CELL DIFFERENTIATION ASSAY

For the identification of ligands specific for HER2, HER3 or HER4, the receptor expression profile of MDA-MB-453 cells offers an excellent indicator for morphologic differentiation inducing activity. This cell line is known to express HER2 and HER3, but contains no detectable EGFR. The results of the semi-quantitative PCR assays (Table III) indicated high level expression of HER4 in MDA-MB-453 cells. In addition, cDNA encoding the prototype HER4 polypeptide of the invention was first isolated from this cell line (Section 6., *supra*).

MDA-MB-453 cells (7500/well) were grown in 50 ml DMEM supplemented with 5% FBS and 1x essential amino acids. Cells were allowed to adhere to 96-well plates for 24 hr. Samples were diluted in the above medium, added to the cell monolayer in 50 ml final volume, and the incubation continued for an additional 3 days. Cells were then examined by inverted light microscopy for morphologic changes.

9.1.2. SOURCE CELLS

Serum free media from a panel of cultures human cancer cells were screened for growth regulatory activity on MDA-MB-453 cells. A human hepatocarcinoma cell line, HepG2, was identified as a source of a factor which induced dramatic morphologic differentiation of the MDA-MB-453 cells.

9.1.3. PURIFICATION OF HER4 LIGAND

The cell differentiation assay described in Section 10.1.1., *supra*, was used throughout the purification procedure to monitor the column fractions that induce morphological changes in MDA-MB-453 cells. For large-scale production of conditioned medium, HepG2 cells were cultured in DMEM containing 10% fetal bovine serum using Nunc cell factories. At about 70% confluence, cells were washed then incubated with serum-free DMEM. Conditioned medium (HepG2-CM) was collected 3 days later, and fresh serum-free medium added to the cells. Two additional harvests of HepG2-CM were collected per cell factory. The medium was centrifuged and stored at -20°C in the presence of 500 mM PMSF.

Ten litres of HepG2-CM were concentrated 16-fold using an Amicon ultrafiltration unit (10,000 molecular weight cutoff membrane), and subjected to sequential precipitation with 20% and 60% ammonium sulfate. After centrifugation at 15,000 x g, the supernatant was extensively dialyzed against PBS and passed through a DEAE-sepharose (Pharmacia) column pre-equilibrated with PBS. The flow-through fraction was then applied onto a 4 ml heparin-acrylic (Bio-Rad) column equilibrated with PBS. Differentiation inducing activity eluted from the heparin column between 0.4 and 0.8 M NaCl. Active heparin fractions were pooled, brought to 2.0 M ammonium sulfate, centrifuged at 12,000 x g for 5 min, and the resulting supernatant was loaded onto a phenyl-5PW column (8 x 75 mm, Waters). Bound proteins were eluted with a decreasing gradient from 2.0 M ammonium sulfate in 0.1 M Na₂HPO₄, pH 7.4 to 0.1 M Na₂HPO₄. Dialyzed fractions were assayed for tyrosine phosphorylation of MDA-MB-453 cells, essentially as described (Wen et al., 1992, Cell 69: 559-72), except PY20 was used as the primary antibody and horseradish peroxidase-conjugated goat F(ab)₂ anti-mouse Ig (Cappel) and chemiluminescence were used for detection. Phosphorylation signals were analyzed using the Molecular Dynamics personal densitometer.

9.2. RESULTS

Semi-purified HepG2-derived factor demonstrated a capacity to induce differentiation in MDA-MB-453 cells (FIG. 9). With reference to the micrographs shown in FIG. 9, untreated MDA-MB-453 cells are moderately adherent and show a rounded morphology (FIG. 9A). In contrast, the addition of semi-purified HepG2-derived factor induces these cells to display a noticeably flattened morphology with larger nuclei and increased cytoplasm (FIG. 9B and 9C). This HepG2-derived factor preparation also binds to heparin, a property which was utilized for purifying the activity.

On further purification, the HepG2-derived factor was found to elute from a phenyl hydrophobic interaction column at 1.0M ammonium sulfate (fractions 16 to 18). FIG. 9D shows the phenyl column elution profile. Tyrosine phosphorylation assays of the phenyl column fractions revealed that the same fractions found to induce differentiation of the human breast carcinoma cells are also able to stimulate tyrosine phosphorylation of a 185 K protein in MDA-MB-453 cells (FIG. 9E). In particular, fraction 16 induced a 4.5-fold increase in the phosphorylation signal compared to the baseline signal observed in unstimulated cells, as determined by densitometry analysis (FIG. 9F).

The phenyl fractions were also tested against the panel of cell lines which each overexpress a single member of the EGFR-family (Section 9.1., *supra*). Fraction 17 induced a significant and specific activation of the HER4 kinase (FIG. 9A, lane 4) without directly affecting the phosphorylation of HER2, EGFR, or HER3 (FIGS. 9B, 9C, and 9D, lane 4). Adjacent fraction 14 was used as a control and had no effect on the phosphorylation of any of the EGFR-family receptors (FIGS. 9A, B, C, D, lane 5). Further purification and analysis of the factor present in fraction 17 indicates that it is a glycoprotein of 40 to 45 kDa, approximately the same size as NDF and HRG. The HepG2-derived factor also has functional properties similar to NDF and HRG, inasmuch as it stimulates tyrosine phosphorylation of HER2/p185 in MDA-MB-453 cells, but not EGFR in NR5 cells, and induces morphologic differentiation of HER2 overexpressing human breast cancer cells.

Recently, several groups have reported the identification of specific ligands for HER2 (see Section 2., *supra*), including NDF and HRG- α . In contrast to these molecules, the HepG2-derived factor described herein failed to stimulate phosphorylation of HER2 in CHO/HER2 cells, but did stimulate phosphorylation of HER4 in CHO/HER4 cells. These findings are intriguing in view of the ability of the HepG2-derived factor to stimulate phosphorylation of MDA-MB-453 cells, a cell line known to overexpress HER2 and HER3 and the source from which HER4 was cloned. Since EGFR and HER2 have been shown to act synergistically, it is conceivable that HER4 may also interact with other EGFR-family members. In this connection, these results suggest that NDF may bind to HER4 in MDA-MB-453 cells resulting in the activation of HER2. The results described in Section 10., immediately below, provide evidence that NDF interacts directly with HER4, resulting in activation of HER2.

10. EXAMPLE: RECOMBINANT NDF-INDUCED, HER4 MEDIATED PHOSPHORYLATION OF HER2

Recombinant NDF was expressed in COS cells and tested for its activity on HER4 in an assay system essentially devoid of other known members of the EGFR-family, notably EGFR and HER2.

A full length rat NDF cDNA was isolated from normal rat kidney RNA and inserted into a cDM8-based expression vector to generate cNDF1.6. This construct was transiently expressed in COS cells, and conditioned cell supernatants were tested for NDF activity using the tyrosine kinase stimulation assay described in Section 8.2., *supra*. Supernatants from cNDF1.6 transfected cells upregulated tyrosine

phosphorylation in MDA-MB-453 cells relative to mock transfected COS media FIG. 10A. Phosphorylation peaked 10-15 minutes after addition on NDF.

The crude NDF supernatants were also tested for the ability to phosphorylate EGFR (NR5 cells), HER2 (CHO/HER2 1-2500 cells), and HER4 (CHO/HER4 21-2 cells). The NDF preparation had no effect on phosphorylation of EGFR, or HER2 containing cells, but induced a 2.4 to 4 fold increase in tyrosine phosphorylation of HER4 after 15 minutes incubation (see FIG. 10B). These findings provide preliminary evidence that NDF/HRG- α mediate their effects not through direct binding to HER2, but instead by means of a direct interaction with HER4. In cell lines expressing both HER2 and HER4, such as MDA-MB-453 cells and other breast carcinoma cells, binding of NDF to HER4 may stimulate HER2 either by heterodimer formation of these two related transmembrane receptors, or by intracellular crosstalk. Formal proof of the direct interaction between NDF and HER4 will require crosslinking of 125 I-NDF to CHO/HER4 cells and a detailed analysis of its binding characteristics.

11. EXAMPLE: CHROMOSOMAL MAPPING OF THE HER4 GENE

A HER4 cDNA probe corresponding to the 5' portion of the gene (nucleotide positions 34-1303) was used for *in situ* hybridization mapping of the HER4 gene. *In situ* hybridization to metaphase chromosomes from lymphocytes of two normal male donors was conducted using the HER4 probe labeled with 3 H to a specific activity of 2.6×10^7 cpm/ μ g as described (Marth et al, 1988, Proc. Natl. Acad. Sci. U.S.A. 83:7400-04). The final probe concentration was 0.05 μ g/ μ l of hybridization mixture. Slides were exposed for one month. Chromosomes were identified by Q banding.

11.2 RESULTS

A total of 58 metaphase cells with autoradiographic grains were examined. Of the 124 hybridization sites scored, 38 (31%) were located on the distal portion of the long arm of chromosome 2 (FIG. 11). The greatest number of grains (21 grains) was located at band q33, with significant numbers of grains on bands q34 (10 grains) and q35 (7 grains). No significant hybridization on other human chromosomes was detected.

12. MICROORGANISM AND CELL DEPOSITS

The following microorganisms and cell lines have been deposited with the American Type Culture Collection, and have been assigned the following accession numbers:

Microorganism	Plasmid	Accession Number
<i>Escherichia coli</i> SCS-1	pBSHER4Y	69 131
(containing the complete human HER4 coding sequence)		

Cell Lines	Accession Number
CHO/HER4 21-2	CRL 11205

The present invention is not to be limited in scope by the microorganisms and cell lines deposited or the embodiments disclosed herein, which are intended as single illustrations of one aspect of the invention, and any which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims. All base pair and amino acid residue numbers and sizes given for polynucleotides and polypeptides are approximate and used for the purpose of description.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: BRISTOL-MYERS SQUIBB COMPANY
 (B) STREET: 345 Park Avenue
 (C) CITY: New York
 (D) STATE: New York
 (E) COUNTRY: U.S.A.
 (F) POSTAL CODE (ZIP): 10154

(ii) TITLE OF INVENTION: HER4 HUMAN RECEPTOR TYROSINE KINASE

(iii) NUMBER OF SEQUENCES: 30

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5501 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 34..3961

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	AATTGTCAGC	ACGGGATCTG	AGACTTCCAA	AAA	ATG	AAG	CCG	GCG	ACA	GGA	CTT	54					
					Met	Lys	Pro	Ala	Thr	Gly	Leu						
					1					5							
5	TGG	GTC	TGG	GTG	AGC	CTT	CTC	GTG	GCG	GCG	GGG	ACC	GTC	CAG	CCC	AGC	102
	Trp	Val	Trp	Val	Ser	Leu	Leu	Val	Ala	Ala	Gly	Thr	Val	Gln	Pro	Ser	
			10					15					20				
	GAT	TCT	CAG	TCA	GTG	TGT	GCA	GGA	ACG	GAG	AAT	AAA	CTG	AGC	TCT	CTC	150
10	Asp	Ser	Gln	Ser	Val	Cys	Ala	Gly	Thr	Glu	Asn	Lys	Leu	Ser	Ser	Leu	
			25				30					35					
	TCT	GAC	CTG	GAA	CAG	CAG	TAC	CGA	GCC	TTG	CGC	AAG	TAC	TAT	GAA	AAC	198
	Ser	Asp	Leu	Glu	Gln	Gln	Tyr	Arg	Ala	Leu	Arg	Lys	Tyr	Tyr	Glu	Asn	
			40			45					50					55	
15	TGT	GAG	GTT	GTG	ATG	GGC	AAC	CTG	GAG	ATA	ACC	AGC	ATT	GAG	CAC	AAC	246
	Cys	Glu	Val	Val	Met	Gly	Asn	Leu	Glu	Ile	Thr	Ser	Ile	Glu	His	Asn	
					60					65					70		
	CGG	GAC	CTC	TCC	TTC	CTG	CGG	TCT	GTT	CGA	GAA	GTC	ACA	GGC	TAC	GTG	294
20	Arg	Asp	Leu	Ser	Phe	Leu	Arg	Ser	Val	Arg	Glu	Val	Thr	Gly	Tyr	Val	
				75				80						85			
	TTA	GTG	GCT	CTT	AAT	CAG	TTT	CGT	TAC	CTG	CCT	CTG	GAG	AAT	TTA	CGC	342
	Leu	Val	Ala	Leu	Asn	Gln	Phe	Arg	Tyr	Leu	Pro	Leu	Glu	Asn	Leu	Arg	
			90					95					100				
25	ATT	ATT	CGT	GGG	ACA	AAA	CTT	TAT	GAG	GAT	CGA	TAT	GCC	TTG	GCA	ATA	390
	Ile	Ile	Arg	Gly	Thr	Lys	Leu	Tyr	Glu	Asp	Arg	Tyr	Ala	Leu	Ala	Ile	
			105				110					115					
	TTT	TTA	AAC	TAC	AGA	AAA	GAT	GGA	AAC	TTT	GGA	CTT	CAA	GAA	CTT	GGA	438
30	Phe	Leu	Asn	Tyr	Arg	Lys	Asp	Gly	Asn	Phe	Gly	Leu	Gln	Glu	Leu	Gly	
			120			125					130					135	
	TTA	AAG	AAC	TTG	ACA	GAA	ATC	CTA	AAT	GGT	GGA	GTC	TAT	GTA	GAC	CAG	486
	Leu	Lys	Asn	Leu	Thr	Glu	Ile	Leu	Asn	Gly	Gly	Val	Tyr	Val	Asp	Gln	
				140						145					150		
35	AAC	AAA	TTC	CTT	TGT	TAT	GCA	GAC	ACC	ATT	CAT	TGG	CAA	GAT	ATT	GTT	534
	Asn	Lys	Phe	Leu	Cys	Tyr	Ala	Asp	Thr	Ile	His	Trp	Gln	Asp	Ile	Val	
				155					160					165			
	CGG	AAC	CCA	TGG	CCT	TCC	AAC	TTG	ACT	CTT	GTG	TCA	ACA	AAT	GGT	AGT	582
40	Arg	Asn	Pro	Trp	Pro	Ser	Asn	Leu	Thr	Leu	Val	Ser	Thr	Asn	Gly	Ser	
			170					175					180				
	TCA	GGA	TGT	GGA	CGT	TGC	CAT	AAG	TCC	TGT	ACT	GGC	CGT	TGC	TGG	GGA	630
	Ser	Gly	Cys	Gly	Arg	Cys	His	Lys	Ser	Cys	Thr	Gly	Arg	Cys	Trp	Gly	
			185			190						195					
45	CCC	ACA	GAA	AAT	CAT	TGC	CAG	ACT	TTG	ACA	AGG	ACG	GTG	TGT	GCA	GAA	678
	Pro	Thr	Glu	Asn	His	Cys	Gln	Thr	Leu	Thr	Arg	Thr	Val	Cys	Ala	Glu	
			200			205					210					215	

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	CAA TGT GAC GGC AGA TGC TAC GGA CCT TAC GTC AGT GAC TGC TGC CAT	726
	Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His	
	220 225 230	
5	CGA GAA TGT GCT GGA GGC TGC TCA GGA CCT AAG GAC ACA GAC TGC TTT	774
	Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe	
	235 240 245	
10	GCC TGC ATG AAT TTC AAT GAC AGT GGA GCA TGT GTT ACT CAG TGT CCC	822
	Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro	
	250 255 260	
	CAA ACC TTT GTC TAC AAT CCA ACC ACC TTT CAA CTG GAG CAC AAT TTC	870
	Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe	
	265 270 275	
15	AAT GCA AAG TAC ACA TAT GGA GCA TTC TGT GTC AAG AAA TGT CCA CAT	918
	Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His	
	280 285 290 295	
20	AAC TTT GTG GTA GAT TCC AGT TCT TGT GTG CGT GCC TGC CCT AGT TCC	966
	Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser	
	300 305 310	
	AAG ATG GAA GTA GAA GAA AAT GGG ATT AAA ATG TGT AAA CCT TGC ACT	1014
	Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr	
	315 320 325	
25	GAC ATT TGC CCA AAA GCT TGT GAT GGC ATT GGC ACA GGA TCA TTG ATG	1062
	Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met	
	330 335 340	
30	TCA GCT CAG ACT GTG GAT TCC AGT AAC ATT GAC AAA TTC ATA AAC TGT	1110
	Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys	
	345 350 355	
	ACC AAG ATC AAT GGG AAT TTG ATC TTT CTA GTC ACT GGT ATT CAT GGG	1158
	Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly	
	360 365 370 375	
35	GAC CCT TAC AAT GCA ATT GAA GCC ATA GAC CCA GAG AAA CTG AAC GTC	1206
	Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val	
	380 385 390	
40	TTT CGG ACA GTC AGA GAG ATA ACA GGT TTC CTG AAC ATA CAG TCA TGG	1254
	Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp	
	395 400 405	
	CCA CCA AAC ATG ACT GAC TTC AGT GTT TTT TCT AAC CTG GTG ACC ATT	1302
	Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile	
	410 415 420	
45	GGT GGA AGA GTA CTC TAT AGT GGC CTG TCC TTG CTT ATC CTC AAG CAA	1350
	Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln	
	425 430 435	

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	CAG	GGC	ATC	ACC	TCT	CTA	CAG	TTC	CAG	TCC	CTG	AAG	GAA	ATC	AGC	GCA	1398
	Gln	Gly	Ile	Thr	Ser	Leu	Gln	Phe	Gln	Ser	Leu	Lys	Glu	Ile	Ser	Ala	
	440					445					450					455	
5	GGA	AAC	ATC	TAT	ATT	ACT	GAC	AAC	AGC	AAC	CTG	TGT	TAT	TAT	CAT	ACC	1446
	Gly	Asn	Ile	Tyr	Ile	Thr	Asp	Asn	Ser	Asn	Leu	Cys	Tyr	Tyr	His	Thr	
					460					465					470		
10	ATT	AAC	TGG	ACA	ACA	CTC	TTC	AGC	ACA	ATC	AAC	CAG	AGA	ATA	GTA	ATC	1494
	Ile	Asn	Trp	Thr	Thr	Leu	Phe	Ser	Thr	Ile	Asn	Gln	Arg	Ile	Val	Ile	
				475					480					485			
	CGG	GAC	AAC	AGA	AAA	GCT	GAA	AAT	TGT	ACT	GCT	GAA	GGA	ATG	GTG	TGC	1542
	Arg	Asp	Asn	Arg	Lys	Ala	Glu	Asn	Cys	Thr	Ala	Glu	Gly	Met	Val	Cys	
			490					495					500				
15	AAC	CAT	CTG	TGT	TCC	AGT	GAT	GGC	TGT	TGG	GGA	CCT	GGG	CCA	GAC	CAA	1590
	Asn	His	Leu	Cys	Ser	Ser	Asp	Gly	Cys	Trp	Gly	Pro	Gly	Pro	Asp	Gln	
		505					510					515					
	TGT	CTG	TCG	TGT	CGC	CGC	TTC	AGT	AGA	GGA	AGG	ATC	TGC	ATA	GAG	TCT	1638
	Cys	Leu	Ser	Cys	Arg	Arg	Phe	Ser	Arg	Gly	Arg	Ile	Cys	Ile	Glu	Ser	
	520				525					530					535		
20	TGT	AAC	CTC	TAT	GAT	GGT	GAA	TTT	CGG	GAG	TTT	GAG	AAT	GGC	TCC	ATC	1686
	Cys	Asn	Leu	Tyr	Asp	Gly	Glu	Phe	Arg	Glu	Phe	Glu	Asn	Gly	Ser	Ile	
					540					545					550		
25	TGT	GTG	GAG	TGT	GAC	CCC	CAG	TGT	GAG	AAG	ATG	GAA	GAT	GGC	CTC	CTC	1734
	Cys	Val	Glu	Cys	Asp	Pro	Gln	Cys	Glu	Lys	Met	Glu	Asp	Gly	Leu	Leu	
				555					560					565			
	ACA	TGC	CAT	GGA	CCG	GGT	CCT	GAC	AAC	TGT	ACA	AAG	TGC	TCT	CAT	TTT	1782
	Thr	Cys	His	Gly	Pro	Gly	Pro	Asp	Asn	Cys	Thr	Lys	Cys	Ser	His	Phe	
			570					575					580				
30	AAA	GAT	GGC	CCA	AAC	TGT	GTG	GAA	AAA	TGT	CCA	GAT	GGC	TTA	CAG	GGG	1830
	Lys	Asp	Gly	Pro	Asn	Cys	Val	Glu	Lys	Cys	Pro	Asp	Gly	Leu	Gln	Gly	
		585					590					595					
	GCA	AAC	AGT	TTC	ATT	TTC	AAG	TAT	GCT	GAT	CCA	GAT	CGG	GAG	TGC	CAC	1878
	Ala	Asn	Ser	Phe	Ile	Phe	Lys	Tyr	Ala	Asp	Pro	Asp	Arg	Glu	Cys	His	
	600					605					610				615		
	CCA	TGC	CAT	CCA	AAC	TGC	ACC	CAA	GGG	TGT	AAC	GGT	CCC	ACT	AGT	CAT	1926
	Pro	Cys	His	Pro	Asn	Cys	Thr	Gln	Gly	Cys	Asn	Gly	Pro	Thr	Ser	His	
					620					625					630		
40	GAC	TGC	ATT	TAC	TAC	CCA	TGG	ACG	GGC	CAT	TCC	ACT	TTA	CCA	CAA	CAT	1974
	Asp	Cys	Ile	Tyr	Tyr	Pro	Trp	Thr	Gly	His	Ser	Thr	Leu	Pro	Gln	His	
				635					640					645			
	GCT	AGA	ACT	CCC	CTG	ATT	GCA	GCT	GGA	GTA	ATT	GGT	GGG	CTC	TTC	ATT	2022
	Ala	Arg	Thr	Pro	Leu	Ile	Ala	Ala	Gly	Val	Ile	Gly	Gly	Leu	Phe	Ile	
			650					655					660				
45																	
50																	
55																	

	CTG	GTC	ATT	GTG	GGT	CTG	ACA	TTT	GCT	GTT	TAT	GTT	AGA	AGG	AAG	AGC	2070
	Leu	Val	Ile	Val	Gly	Leu	Thr	Phe	Ala	Val	Tyr	Val	Arg	Arg	Lys	Ser	
	665					670						675					
5	ATC	AAA	AAG	AAA	AGA	GCC	TTG	AGA	AGA	TTC	TTG	GAA	ACA	GAG	TTG	GTG	2118
	Ile	Lys	Lys	Lys	Arg	Ala	Leu	Arg	Arg	Phe	Leu	Glu	Thr	Glu	Leu	Val	
	680					685					690					695	
	GAA	CCA	TTA	ACT	CCC	AGT	GGC	ACA	GCA	CCC	AAT	CAA	GCT	CAA	CTT	CGT	2166
	Glu	Pro	Leu	Thr	Pro	Ser	Gly	Thr	Ala	Pro	Asn	Gln	Ala	Gln	Leu	Arg	
					700					705					710		
10	ATT	TTG	AAA	GAA	ACT	GAG	CTG	AAG	AGG	GTA	AAA	GTC	CTT	GGC	TCA	GGT	2214
	Ile	Leu	Lys	Glu	Thr	Glu	Leu	Lys	Arg	Val	Lys	Val	Leu	Gly	Ser	Gly	
				715					720					725			
	GCT	TTT	GGA	ACG	GTT	TAT	AAA	GGT	ATT	TGG	GTA	CCT	GAA	GGA	GAA	ACT	2262
15	Ala	Phe	Gly	Thr	Val	Tyr	Lys	Gly	Ile	Trp	Val	Pro	Glu	Gly	Glu	Thr	
			730					735					740				
	GTG	AAG	ATT	CCT	GTG	GCT	ATT	AAG	ATT	CTT	AAT	GAG	ACA	ACT	GGT	CCC	2310
	Val	Lys	Ile	Pro	Val	Ala	Ile	Lys	Ile	Leu	Asn	Glu	Thr	Thr	Gly	Pro	
		745					750					755					
20	AAG	GCA	AAT	GTG	GAG	TTC	ATG	GAT	GAA	GCT	CTG	ATC	ATG	GCA	AGT	ATG	2358
	Lys	Ala	Asn	Val	Glu	Phe	Met	Asp	Glu	Ala	Leu	Ile	Met	Ala	Ser	Met	
	760					765				770						775	
	GAT	CAT	CCA	CAC	CTA	GTC	CGG	TTG	CTG	GGT	GTG	TGT	CTG	AGC	CCA	ACC	2406
25	Asp	His	Pro	His	Leu	Val	Arg	Leu	Leu	Gly	Val	Cys	Leu	Ser	Pro	Thr	
					780					785					790		
	ATC	CAG	CTG	GTT	ACT	CAA	CTT	ATG	CCC	CAT	GGC	TGC	CTG	TTG	GAG	TAT	2454
	Ile	Gln	Leu	Val	Thr	Gln	Leu	Met	Pro	His	Gly	Cys	Leu	Leu	Glu	Tyr	
				795					800					805			
30	GTC	CAC	GAG	CAC	AAG	GAT	AAC	ATT	GGA	TCA	CAA	CTG	CTG	CTT	AAC	TGG	2502
	Val	His	Glu	His	Lys	Asp	Asn	Ile	Gly	Ser	Gln	Leu	Leu	Leu	Asn	Trp	
			810					815					820				
	TGT	GTC	CAG	ATA	GCT	AAG	GGA	ATG	ATG	TAC	CTG	GAA	GAA	AGA	CGA	CTC	2550
35	Cys	Val	Gln	Ile	Ala	Lys	Gly	Met	Met	Tyr	Leu	Glu	Glu	Arg	Arg	Leu	
		825					830					835					
	GTT	CAT	CGG	GAT	TTG	GCA	GCC	CGT	AAT	GTC	TTA	GTG	AAA	TCT	CCA	AAC	2598
	Val	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Val	Leu	Val	Lys	Ser	Pro	Asn	
	840				845					850						855	
40	CAT	GTG	AAA	ATC	ACA	GAT	TTT	GGG	CTA	GCC	AGA	CTC	TTG	GAA	GGA	GAT	2646
	His	Val	Lys	Ile	Thr	Asp	Phe	Gly	Leu	Ala	Arg	Leu	Leu	Glu	Gly	Asp	
					860					865					870		
	GAA	AAA	GAG	TAC	AAT	GCT	GAT	GGA	GGA	AAG	ATG	CCA	ATT	AAA	TGG	ATG	2694
45	Glu	Lys	Glu	Tyr	Asn	Ala	Asp	Gly	Gly	Lys	Met	Pro	Ile	Lys	Trp	Met	
				875				880							885		
50																	
55																	

	GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT GAC GTT	2742
	Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val	
	890 895 900	
5	TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA GGA AAA	2790
	Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys	
	905 910 915	
	CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA GAG AAA	2838
	Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys	
	920 925 930 935	
10	GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT TAC ATG	2886
	Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met	
	940 945 950	
	GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT AAA TTT	2934
	Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe	
	955 960 965	
	AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT CAA AGA	2982
	Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg	
	970 975 980	
20	TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT CCA AAT	3030
	Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn	
	985 990 995	
	GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG GAA GAT	3078
	Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp	
	1000 1005 1010 1015	
	ATG ATG GAT GCT GAG GAG TAC TTG GTC CCT CAG GCT TTC AAC ATC CCA	3126
	Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro	
	1020 1025 1030	
30	CCT CCC ATC TAT ACT TCC AGA GCA AGA ATT GAC TCG AAT AGG AGT GAA	3174
	Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Glu	
	1035 1040 1045	
	ATT GGA CAC AGC CCT CCT CCT GCC TAC ACC CCC ATG TCA GGA AAC CAG	3222
	Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly Asn Gln	
	1050 1055 1060	
	TTT GTA TAC CGA GAT GGA GGT TTT GCT GCT GAA CAA GGA GTG TCT GTG	3270
	Phe Val Tyr Arg Asp Gly Gly Phe Ala Ala Glu Gln Gly Val Ser Val	
	1065 1070 1075	
40	CCC TAC AGA GCC CCA ACT AGC ACA ATT CCA GAA GCT CCT GTG GCA CAG	3318
	Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val Ala Gln	
	1080 1085 1090 1095	
	GGT GCT ACT GCT GAG ATT TTT GAT GAC TCC TGC TGT AAT GGC ACC CTA	3366
	Gly Ala Thr Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly Thr Leu	
	1100 1105 1110	
45		
50		
55		

	CGC AAG CCA GTG GCA CCC CAT GTC CAA GAG GAC AGT AGC ACC CAG AGG	3414
	Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr Gln Arg	
	1115 1120 1125	
5	TAC AGT GCT GAC CCC ACC GTG TTT GCC CCA GAA CGG AGC CCA CGA GGA	3462
	Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro Arg Gly	
	1130 1135 1140	
	GAG CTG GAT GAG GAA GGT TAC ATG ACT CCT ATG CGA GAC AAA CCC AAA	3510
10	Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys Pro Lys	
	1145 1150 1155	
	CAA GAA TAC CTG AAT CCA GTG GAG GAG AAC CCT TTT GTT TCT CGG AGA	3558
	Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser Arg Arg	
	1160 1165 1170 1175	
15	AAA AAT GGA GAC CTT CAA GCA TTG GAT AAT CCC GAA TAT CAC AAT GCA	3606
	Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His Asn Ala	
	1180 1185 1190	
	TCC AAT GGT CCA CCC AAG GCC GAG GAT GAG TAT GTG AAT GAG CCA CTG	3654
20	Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu Pro Leu	
	1195 1200 1205	
	TAC CTC AAC ACC TTT GCC AAC ACC TTG GGA AAA GCT GAG TAC CTG AAG	3702
	Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr Leu Lys	
	1210 1215 1220	
25	AAC AAC ATA CTG TCA ATG CCA GAG AAG GCC AAG AAA GCG TTT GAC AAC	3750
	Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe Asp Asn	
	1225 1230 1235	
	CCT GAC TAC TGG AAC CAC AGC CTG CCA CCT CGG AGC ACC CTT CAG CAC	3798
30	Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu Gln His	
	1240 1245 1250 1255	
	CCA GAC TAC CTG CAG GAG TAC AGC ACA AAA TAT TTT TAT AAA CAG AAT	3846
	Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys Gln Asn	
	1260 1265 1270	
35	GGG CGG ATC CGG CCT ATT GTG GCA GAG AAT CCT GAA TAC CTC TCT GAG	3894
	Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu Ser Glu	
	1275 1280 1285	
	TTC TCC CTG AAG CCA GGC ACT GTG CTG CCG CCT CCA CCT TAC AGA CAC	3942
40	Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Tyr Arg His	
	1290 1295 1300	
	CGG AAT ACT GTG GTG TAAGCTCAGT TGTGGTTTTT TAGGTGGAGA GACACACCTG	3997
	Arg Asn Thr Val Val	
	1305	
45	CTCCAATTTC CCCACCCCCC TCTCTTTCTC TGGTGGTCTT CCTTCTACCC CAAGGCCAGT	4057
	AGTTTTGACA CTCCCAGTG GAAGATACAG AGATGCAATG ATAGTTATGT GCTTACCTAA	4117
	CTTGAACATT AGAGGGAAAG ACTGAAAGAG AAAGATAGGA GGAACCACAA TGTTTCTTCA	4177
50		
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TTTCTCTGCA TGGGTTGGTC AGGAGAATGA AACAGCTAGA GAAGGACCAG AAAATGTAAG 4237
 GCAATGCTGC CTACTATCAA ACTAGCTGTC ACTTTTTTTC TTTTCTTTT TCTTCTTTG 4297
 5 TTTCTTTCTT CTTCTCTTT TTTTTTTTT TTTTAAAGCA GATGTTGAA ACACCCATGC 4357
 TATCTGTTCC TATCTGCAGG AACTGATGTG TGCATATTTA GCATCCCTGG AAATCATAAT 4417
 AAAGTTTCCA TTAGAACAAA AGAATAACAT TTTCTATAAC ATATGATAGT GTCTGAAATT 4477
 10 GAGAATCCAG TTTCTTTCCC CAGCAGTTTC TGTCTAGCA AGTAAGAATG GCCAACTCAA 4537
 CTTTCATAAT TTAAAAATCT CCATTAAAGT TATAACTAGT AATTATGTTT TCAACACTTT 4597
 TTGGTTTTTT TCAATTTGTT TTGCTCTGAC CGATTCCTTT ATATTGCTC CCCTATTTTT 4657
 15 GGCTTTAATT TCTAATTGCA AAGATGTTTA CATCAAAGCT TCTTCACAGA ATTTAAGCAA 4717
 GAAATATTTT AATATAGTGA AATGGCCACT ACTTTAAGTA TACAATCTTT AAAATAAGAA 4777
 AGGGAGGCTA ATATTTTTC TACTATCAAA TTATCTTCAC CCTCATCCTT TACATTTTTT 4837
 AACATTTTTT TTTCTCCATA AATGACACTA CTTGATAGGC CGTTGGTTGT CTGAAGAGTA 4897
 20 GAAGGGAAC TAAGAGACAG TTCTCTGTGG TTCAGGAAAA CTACTGATAC TTTCAGGGGT 4957
 GGCCCAATGA GGGAAATCCAT TGAAGTGGAA GAAACACACT GGATTGGGTA TGTCTACCTG 5017
 GCAGATACTC AGAAATGTAG TTTGCACTTA AGCTGTAATT TTATTGTTC TTTTCTGAA 5077
 25 CTCCATTTTG GATTTTGAAT CAAGCAATAT GGAAGCAACC AGCAAATTAA CTAATTTAAG 5137
 TACATTTTTA AAAAAAGAGC TAAGATAAAG ACTGTGGAAA TGCCAAACCA AGCAAATTAG 5197
 GAACCTTGCA ACGGTATCCA GGGACTATGA TGAGAGGCCA GCACATTATC TTCATATGTC 5257
 30 ACCTTTGCTA CGCAAGGAAA TTTGTTCACT TCGTATACTT CGTAAGAAGG AATGCGAGTA 5317
 AGGATTGGCT TGAATTCCAT GGAATTTCTA GTATGAGACT ATTTATATGA AGTAGAAGGT 5377
 AACTCTTTGC ACATAAATTG GTATAATAAA AAGAAAAACA CAAACATTCA AAGCTTAGGG 5437
 35 ATAGGTCCTT GGGTCAAAAG TTGTAAATAA ATGTGAAACA TCTTCTCAA AAAAAAAAAA 5497
 AAAA 5501

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1308 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1 5 10 15
 6 Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
 20 25 30
 Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
 35 40 45
 10 Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
 50 55 60
 Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
 65 70 75 80
 15 Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
 85 90 95
 Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
 100 105 110
 20 Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
 115 120 125
 Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
 130 135 140
 25 Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
 145 150 155 160
 Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
 165 170 175
 30 Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
 180 185 190
 Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
 195 200 205
 35 Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
 210 215 220
 Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
 225 230 235 240
 Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
 245 250 255
 45 Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
 260 265 270
 Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
 275 280 285
 50 Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
 290 295 300

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Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
 305 310 315 320
 5 Lys Met Cys Lys Pr Cys Thr Asp Ile Cys Pr Lys Ala Cys Asp Gly
 325 330 335
 Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
 340 345 350
 10 Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
 355 360 365
 Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
 370 375 380
 15 Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
 385 390 395 400
 Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
 405 410 415
 20 Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
 420 425 430
 Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
 435 440 445
 25 Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
 450 455 460
 Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
 465 470 475 480
 30 Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
 485 490 495
 Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
 500 505 510
 35 Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
 515 520 525
 Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
 530 535 540
 40 Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
 545 550 555 560
 Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
 565 570 575
 45 Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
 580 585 590
 50 Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
 595 600 605

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Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
 610 615 620
 Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
 625 630 635 640
 His Ser Thr Leu Pro Gln His Ala Arg Thr Pro Leu Ile Ala Ala Gly
 645 650 655
 Val Ile Gly Gly Leu Phe Ile Leu Val Ile Val Gly Leu Thr Phe Ala
 660 665 670
 Val Tyr Val Arg Arg Lys Ser Ile Lys Lys Lys Arg Ala Leu Arg Arg
 675 680 685
 Phe Leu Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Thr Ala
 690 695 700
 Pro Asn Gln Ala Gln Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg
 705 710 715 720
 Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile
 725 730 735
 Trp Val Pro Glu Gly Glu Thr Val Lys Ile Pro Val Ala Ile Lys Ile
 740 745 750
 Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu
 755 760 765
 Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu
 770 775 780
 Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro
 785 790 795 800
 His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly
 805 810 815
 Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met
 820 825 830
 Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn
 835 840 845
 Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu
 850 855 860
 Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly
 865 870 875 880
 Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys
 885 890 895
 Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu
 900 905 910

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Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pr Thr Arg Glu
 915 920 925
 Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
 930 935 940
 Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
 945 950 955 960
 Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
 965 970 975
 Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
 980 985 990
 Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
 995 1000 1005
 Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
 1010 1015 1020
 Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
 1025 1030 1035 1040
 Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr
 1045 1050 1055
 Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe Ala
 1060 1065 1070
 Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile
 1075 1080 1085
 Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp Asp
 1090 1095 1100
 Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val Gln
 1105 1110 1115 1120
 Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe Ala
 1125 1130 1135
 Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met Thr
 1140 1145 1150
 Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu Glu
 1155 1160 1165
 Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu Asp
 1170 1175 1180
 Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu Asp
 1185 1190 1195 1200
 Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr Leu
 1205 1210 1215

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Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu Lys
 1220 1225 1230
 5 Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro
 1235 1240 1245
 Pro Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr
 1250 1255 1260
 10 Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu
 1265 1270 1275 1280
 Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu
 1285 1290 1295
 15 Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
 1300 1305

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5555 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 34..3210

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTGTCAGC ACGGGATCTG AGACTTCCAA AAA ATG AAG CCG GCG ACA GGA CTT 54
 Met Lys Pro Ala Thr Gly Leu
 1 5
 35 TGG GTC TGG GTG AGC CTT CTC GTG GCG GCG GGG ACC GTC CAG CCC AGC 102
 Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
 10 15 20
 GAT TCT CAG TCA GTG TGT GCA GGA ACG GAG AAT AAA CTG AGC TCT CTC 150
 Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
 40 25 30 35
 TCT GAC CTG GAA CAG CAG TAC CGA GCC TTG CGC AAG TAC TAT GAA AAC 198
 Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn
 40 45 50 55
 45 TGT GAG GTT GTC ATG GGC AAC CTG GAG ATA ACC AGC ATT GAG CAC AAC 246
 Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn
 60 65 70

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	CGG GAC CTC TCC TTC CTG CGG TCT GTT CGA GAA GTC ACA GGC TAC GTC	294
	Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val	
	75 80 85	
5	TTA GTG GCT CTT AAT CAG TTT CGT TAC CTG CCT CTG GAG AAT TTA CGC	342
	Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg	
	90 95 100	
	ATT ATT CGT GGG ACA AAA CTT TAT GAG GAT CGA TAT GCC TTG GCA ATA	390
10	Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile	
	105 110 115	
	TTT TTA AAC TAC AGA AAA GAT GGA AAC TTT GGA CTT CAA GAA CTT GGA	438
	Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly	
	120 125 130 135	
15	TTA AAG AAC TTG ACA GAA ATC CTA AAT GGT GGA GTC TAT GTA GAC CAG	486
	Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln	
	140 145 150	
	AAC AAA TTC CTT TGT TAT GCA GAC ACC ATT CAT TGG CAA GAT ATT GTT	534
20	Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val	
	155 160 165	
	CGG AAC CCA TGG CCT TCC AAC TTG ACT CTT GTG TCA ACA AAT GGT AGT	582
	Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser	
	170 175 180	
25	TCA GGA TGT GGA CGT TGC CAT AAG TCC TGT ACT GGC CGT TGC TGG GGA	630
	Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly	
	185 190 195	
	CCC ACA GAA AAT CAT TGC CAG ACT TTG ACA AGG ACG GTG TGT GCA GAA	678
30	Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu	
	200 205 210 215	
	CAA TGT GAC GGC AGA TGC TAC GGA CCT TAC GTC AGT GAC TGC TGC CAT	726
	Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His	
	220 225 230	
35	CGA GAA TGT GCT GGA GGC TGC TCA GGA CCT AAG GAC ACA GAC TGC TTT	774
	Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe	
	235 240 245	
	GCC TGC ATG AAT TTC AAT GAC AGT GGA GCA TGT GTT ACT CAG TGT CCC	822
40	Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro	
	250 255 260	
	CAA ACC TTT GTC TAC AAT CCA ACC ACC TTT CAA CTG GAG CAC AAT TTC	870
	Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe	
	265 270 275	
45	AAT GCA AAG TAC ACA TAT GGA GCA TTC TGT GTC AAG AAA TGT CCA CAT	918
	Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His	
	280 285 290 295	

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	AAC TTT GTG GTA GAT TCC AGT TCT TGT GTG CGT GCC TGC CCT AGT TCC	966
	Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser	
	300 305 310	
6	AAG ATG GAA GTA GAA GAA AAT GGG ATT AAA ATG TGT AAA CCT TGC ACT	1014
	Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr	
	315 320 325	
	GAC ATT TGC CCA AAA GCT TGT GAT GGC ATT GGC ACA GGA TCA TTG ATG	1062
	Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met	
	330 335 340	
10	TCA GCT CAG ACT GTG GAT TCC AGT AAC ATT GAC AAA TTC ATA AAC TGT	1110
	Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys	
	345 350 355	
15	ACC AAG ATC AAT GGG AAT TTG ATC TTT CTA GTC ACT GGT ATT CAT GGG	1158
	Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly	
	360 365 370 375	
	GAC CCT TAC AAT GCA ATT GAA GCC ATA GAC CCA GAG AAA CTG AAC GTC	1206
	Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val	
	380 385 390	
20	TTT CGG ACA GTC AGA GAG ATA ACA GGT TTC CTG AAC ATA CAG TCA TGG	1254
	Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp	
	395 400 405	
25	CCA CCA AAC ATG ACT GAC TTC AGT GTT TTT TCT AAC CTG GTG ACC ATT	1302
	Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile	
	410 415 420	
	GGT GGA AGA GTA CTC TAT AGT GGC CTG TCC TTG CTT ATC CTC AAG CAA	1350
	Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln	
	425 430 435	
30	CAG GGC ATC ACC TCT CTA CAG TTC CAG TCC CTG AAG GAA ATC AGC GCA	1398
	Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala	
	440 445 450 455	
	GGA AAC ATC TAT ATT ACT GAC AAC AGC AAC CTG TGT TAT TAT CAT ACC	1446
	Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr	
	460 465 470	
	ATT AAC TGG ACA ACA CTC TTC AGC ACA ATC AAC CAG AGA ATA GTA ATC	1494
	Ile Asn Trp Thr Thr Leu Phe Ser Thr Ile Asn Gln Arg Ile Val Ile	
	475 480 485	
40	CGG GAC AAC AGA AAA GCT GAA AAT TGT ACT GCT GAA GGA ATG GTG TGC	1542
	Arg Asp Asn Arg Lys Ala Glu Asn Cys Thr Ala Glu Gly Met Val Cys	
	490 495 500	
45	AAC CAT CTG TGT TCC AGT GAT GGC TGT TGG GGA CCT GGG CCA GAC CAA	1590
	Asn His Leu Cys Ser Ser Asp Gly Cys Trp Gly Pro Gly Pro Asp Gln	
	505 510 515	

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	TGT	CTG	TCG	TGT	CGC	CGC	TTC	AGT	AGA	GGA	AGG	ATC	TGC	ATA	GAG	TCT	1638
	Cys	Leu	Ser	Cys	Arg	Arg	Phe	Ser	Arg	Gly	Arg	Ile	Cys	Ile	Glu	Ser	
	520				525					530					535		
5	TGT	AAC	CTC	TAT	GAT	GGT	GAA	TTT	CGG	GAG	TTT	GAG	AAT	GGC	TCC	ATC	1686
	Cys	Asn	Leu	Tyr	Asp	Gly	Glu	Phe	Arg	Glu	Phe	Glu	Asn	Gly	Ser	Ile	
					540					545					550		
	TGT	GTG	GAG	TGT	GAC	CCC	CAG	TGT	GAG	AAG	ATG	GAA	GAT	GGC	CTC	CTC	1734
	Cys	Val	Glu	Cys	Asp	Pro	Gln	Cys	Glu	Lys	Met	Glu	Asp	Gly	Leu	Leu	
10				555					560					565			
	ACA	TGC	CAT	GGA	CCG	GGT	CCT	GAC	AAC	TGT	ACA	AAG	TGC	TCT	CAT	TTT	1782
	Thr	Cys	His	Gly	Pro	Gly	Pro	Asp	Asn	Cys	Thr	Lys	Cys	Ser	His	Phe	
				570				575						580			
15	AAA	GAT	GGC	CCA	AAC	TGT	GTG	GAA	AAA	TGT	CCA	GAT	GGC	TTA	CAG	GGG	1830
	Lys	Asp	Gly	Pro	Asn	Cys	Val	Glu	Lys	Cys	Pro	Asp	Gly	Leu	Gln	Gly	
		585					590					595					
	GCA	AAC	AGT	TTC	ATT	TTC	AAG	TAT	GCT	GAT	CCA	GAT	CGG	GAG	TGC	CAC	1878
	Ala	Asn	Ser	Phe	Ile	Phe	Lys	Tyr	Ala	Asp	Pro	Asp	Arg	Glu	Cys	His	
20		600				605					610					615	
	CCA	TGC	CAT	CCA	AAC	TGC	ACC	CAA	GGG	TGT	AAC	GGT	CCC	ACT	AGT	CAT	1926
	Pro	Cys	His	Pro	Asn	Cys	Thr	Gln	Gly	Cys	Asn	Gly	Pro	Thr	Ser	His	
				620					625						630		
25	GAC	TGC	ATT	TAC	TAC	CCA	TGG	ACG	GGC	CAT	TCC	ACT	TTA	CCA	CAA	CAT	1974
	Asp	Cys	Ile	Tyr	Tyr	Pro	Trp	Thr	Gly	His	Ser	Thr	Leu	Pro	Gln	His	
				635					640					645			
	GCT	AGA	ACT	CCC	CTG	ATT	GCA	GCT	GGA	GTA	ATT	GGT	GGG	CTC	TTC	ATT	2022
	Ala	Arg	Thr	Pro	Leu	Ile	Ala	Ala	Gly	Val	Ile	Gly	Gly	Leu	Phe	Ile	
30				650				655					660				
	CTG	GTG	ATT	GTG	GGT	CTG	ACA	TTT	GCT	GTT	TAT	GTT	AGA	AGG	AAG	AGC	2070
	Leu	Val	Ile	Val	Gly	Leu	Thr	Phe	Ala	Val	Tyr	Val	Arg	Arg	Lys	Ser	
				665				670				675					
35	ATC	AAA	AAG	AAA	AGA	GCC	TTG	AGA	AGA	TTC	TTG	GAA	ACA	GAG	TTG	GTG	2118
	Ile	Lys	Lys	Lys	Arg	Ala	Leu	Arg	Arg	Phe	Leu	Glu	Thr	Glu	Leu	Val	
		680				685					690					695	
	GAA	CCA	TTA	ACT	CCC	AGT	GGC	ACA	GCA	CCC	AAT	CAA	GCT	CAA	CTT	CGT	2166
	Glu	Pro	Leu	Thr	Pro	Ser	Gly	Thr	Ala	Pro	Asn	Gln	Ala	Gln	Leu	Arg	
40				700						705					710		
	ATT	TTG	AAA	GAA	ACT	GAG	CTG	AAG	AGG	GTA	AAA	GTC	CTT	GGC	TCA	GGT	2214
	Ile	Leu	Lys	Glu	Thr	Glu	Leu	Lys	Arg	Val	Lys	Val	Leu	Gly	Ser	Gly	
				715				720						725			
45	GCT	TTT	GGA	ACG	GTT	TAT	AAA	GGT	ATT	TGG	GTA	CCT	GAA	GGA	GAA	ACT	2262
	Ala	Phe	Gly	Thr	Val	Tyr	Lys	Gly	Ile	Trp	Val	Pro	Glu	Gly	Glu	Thr	
			730					735						740			
50																	
55																	

	GTG AAG ATT CCT GTG GCT ATT AAG ATT CTT AAT GAG ACA ACT GGT CCC Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro 745 750 755	2310
5	AAG GCA AAT GTG GAG TTC ATG GAT GAA GCT CTG ATC ATG GCA AGT ATG Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met 760 765 770 775	2358
10	GAT CAT CCA CAC CTA GTC CGG TTG CTG GGT GTG TGT CTG AGC CCA ACC Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr 780 785 790	2406
	ATC CAG CTG GTT ACT CAA CTT ATG CCC CAT GGC TGC CTG TTG GAG TAT Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr 795 800 805	2454
15	GTC CAC GAG CAC AAG GAT AAC ATT GGA TCA CAA CTG CTG CTT AAC TGG Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp 810 815 820	2502
20	TGT GTC CAG ATA GCT AAG GGA ATG ATG TAC CTG GAA GAA ACA CGA CTC Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu 825 830 835	2550
	GTT CAT CGG GAT TTG GCA GCC CGT AAT GTC TTA GTG AAA TCT CCA AAC Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn 840 845 850 855	2598
25	CAT GTG AAA ATC ACA GAT TTT GGG CTA GCC ACA CTC TTG GAA GGA GAT His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp 860 865 870	2646
30	GAA AAA GAG TAC AAT GCT GAT GGA GGA AAG ATG CCA ATT AAA TGG ATG Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met 875 880 885	2694
	GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT GAC GTT Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val 890 895 900	2742
35	TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA GGA AAA Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys 905 910 915	2790
40	CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA GAG AAA Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys 920 925 930 935	2838
	GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT TAC ATG Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met 940 945 950	2886
45	GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT AAA TTT Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe 955 960 965	2934

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	AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT CAA AGA	2982
	Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg	
	970 975 980	
5	TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT CCA AAT	3030
	Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pr Ser Pro Asn	
	985 990 995	
	GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG GAA GAT	3078
	Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp	
10	1000 1005 1010 1015	
	ATG ATG GAT GCT GAG GAG TAC TTG GTC CCT CAG GCT TTC AAC ATC CCA	3126
	Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro	
	1020 1025 1030	
15	CCT CCC ATC TAT ACT TCC AGA GCA AGA ATT GAC TCG AAT AGG AGT GTA	3174
	Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Val	
	1035 1040 1045	
	AGA AAT AAT TAT ATA CAC ATA TCA TAT TCT TTC TGAGATATAA AATCATGTAA	3227
	Arg Asn Asn Tyr Ile His Ile Ser Tyr Ser Phe	
20	1050 1055	
	TAGTTCATAA GCACTAACAT TTCAAATAA TTATATAGCT CAAATCAATG TGATGCCTAG	3287
	ATTAATAATA TACCATACCC ACAAAGATG TGCCAATCTT GCTATATGTA GTTAATTTTG	3347
	GAAGACAAGC ATGGACAATA CAACATGTAC TCTGAAATAC CTTCAAGATT TCAGAAGCAA	3407
25	AACATTTTCC TCATCTTAAT TTATTTAAAA CAAATCTTAA CTTTAAAAAA CAATTCCAAC	3467
	TAATAAAACC ATTATGTGTA TATAAATAAA TGAAAATTCC TACCAAGTAG GCTTTCTACT	3527
	TTTCTTTCTT AAAAAGATAT TATGATATAT TAGTCAAGAA GTAATACAAG TATAAATCTC	3587
30	TTTCACTTAT TTAAGAAAAA TTAAATATTT TCTGTCAAGT TGAAGTAGAA ACACAGAAAA	3647
	CCGTGCAGTC CTTTGAACCT AATCACATCG AAAAGGCTGC TGAGAAGTAG ATTTTGTGTT	3707
	TTAAGAAGTA GATTTAAGTT TTGAAGGAAG TTTCTGAAAA CACTTTACAT TTTAAATGTT	3767
35	AAACCTACTC TATATGAATT CCATTCTTTC TTTGAAAGCT GTCAAATCCA TGCATTTATT	3827
	TTTATAAATT CATTCTCAT ACATTCAACA TATATTGAGT ACCACTGTAT GTGAAGCATT	3887
	AGTATACATT TAAGACTCAA AGAATTTTGA TACAACCTCT GCTTTCAAGA AGTGAAGAAC	3947
40	TTAATCAAAG AATCATACAG ATAGAGGGAC TGCATAGTAA GTGCTGTAAT CCAGTATTCA	4007
	CTGACCAGTA CGGAGCATGA AGAAGTAGTA AATTTGTGTC TGTAATCAGT TTCTTCCATT	4067
	GATAAGATAT AAACATGATG CTTAATTTTT TCTAGAAGAT AATTCTTTTC TCTTAATCTA	4127
45	AGAACATTAT CATAGCTAGT AGAACCGACA GCATCCGATT TCTCTTGACC ATAGCCATAA	4187
	GAATATCTTC AACTTGCTGC TCATTATCTA ACAAACATAA TTTTCTTTAT TTCATATTGA	4247

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TTGTAATAAG TAATATCCCC CTGGAAGTTT ACTATTCAAC ACATATATGT TAACCTCCTT 4307
 AATTCCTTAA ACAAACCTCA TGAGGTCTTA TTATTATCAT CCCCTTCTTT CAAAGGAAGA 4367
 5 AACTTGCCAC AGAGAAGTCA GGTGATATGA CTGGTGTAC ACAGCTAGTC AGTGAAGAG 4427
 AGGAATAAGT AATCTAGATA TCTGCCTACT ACACTGTAGG TTTGCTTCAA AGTTACTGAA 4487
 GYCATGTTAT TTCCATGATG TGATTAGAGT CTGGGACTTG TCTTGTGTTGG GAAATTTCCC 4547
 10 AGGTGGTTTT CTTATAAAAT GCATCTCAA TCTGCTCTAC ACCTTTTACT CATCTACCTC 4607
 CATTTAGAAG ATCTGATATG GAAAGAGACA AAGATGGAGA CCTCAATTAT TTTTCTTTT 4667
 CTGTTAAAAA TATTATAGTA CAACTGAAAC TTATCACATG CCAATGGGGA ATAGATAACT 4727
 15 AAAAGTTTAA AATTAGATCA ATGGATAGGT AAATGAATAA TCNTTCTTTT GCTTGTGAGA 4787
 GGGGAAGGAA AAGCGGTTAA GGTGGTATAA AGGAGGCTCC TCTGTACACT TGCAAAATGA 4847
 TCAAATTATA TACCCTTGTA TTTATAATTT TAAGTGACAA ATTCATTACT TCTGGTTACA 4907
 20 ACAGTGAAAT TAAAAAATA ATAGTTTTTC TTTCTTAGCT TGCAATGCTA TAAATCTTTT 4967
 TCTTTTTATA AGAATTCCTA CATTTACGCT TTTTGTTCAT TTAAATTTAT AATTCTCAGT 5027
 GCAAGAAATT CTTAATAAAG GTTTGAGCTA GCTAGATGGA ATTATTGAGA CAAAGTCTAA 5087
 25 ATCACCCGTG GACTTATTTG ACCTTTAGCC ATCATTCTCT ATTCCACATT ATAAAAAAT 5147
 GTTACCTGTA GATTTCTTTT TACTTTTTCA GTCCTTGGAA AAGAAATGGT GATTAAATAT 5207
 CATTATATCA TTTTATGTTT AGGCATTATA AAAGCTTTAT TTGTCATCTA TATTGTCCTA 5267
 30 ATAGTTTTCA GTCTGGCTTT ACGTAACTTT TACGGAAATT TCTAACATGT ACAAATGCCA 5327
 TGTTCCTCCT TTCTTTCCTA CATGGCTGAA TTAGAAAACA AATTACTTCC ATTTTAAGTT 5387
 TGGCTAAATT AGAAAACAAA TTAATACCAT TTTAAGTTTG GTGGCTAAAT AACGTGCTAA 5447
 35 GGGAACATCT TAAAAAGTGA ATTTTGATCA AATATTTCTT AAGCATATGT GATAGACTTT 5507
 GAAACCAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 5555

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 40 (A) LENGTH: 1058 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1 5 10 15

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Ala Gly Thr Val Gln Pr Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
 20 25 30
 5 Glu Asn Lys Leu S r Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
 35 40 45
 Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
 50 55 60
 10 Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
 65 70 75 80
 Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
 85 90 95
 15 Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
 100 105 110
 Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
 115 120 125
 20 Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
 130 135 140
 Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
 145 150 155 160
 25 Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
 165 170 175
 Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
 180 185 190
 30 Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
 195 200 205
 Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
 210 215 220
 35 Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
 225 230 235 240
 40 Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
 245 250 255
 Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
 260 265 270
 45 Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
 275 280 285
 Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
 290 295 300
 50 Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
 305 310 315 320

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Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
 325 330 335
 5 Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
 340 345 350
 Ile Asp Lys Phe Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
 355 360 365
 10 Leu Val Thr Gly Ile His Gly Asp Pro Tyr Asn Ala Ile Glu Ala Ile
 370 375 380
 Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
 385 390 395 400
 15 Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
 405 410 415
 Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
 420 425 430
 20 Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
 435 440 445
 Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
 450 455 460
 25 Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
 465 470 475 480
 Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
 485 490 495
 30 Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
 500 505 510
 Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
 515 520 525
 35 Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
 530 535 540
 Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
 545 550 555 560
 Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
 565 570 575
 40 Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
 580 585 590
 Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
 595 600 605
 50 Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
 610 615 620

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Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
 625 630 635 640
 5 His Ser Thr Leu Pro Gln His Ala Arg Thr Pro Leu Ile Ala Ala Gly
 645 650 655
 Val Ile Gly Gly Leu Phe Ile Leu Val Ile Val Gly Leu Thr Phe Ala
 660 665 670
 10 Val Tyr Val Arg Arg Lys Ser Ile Lys Lys Lys Arg Ala Leu Arg Arg
 675 680 685
 Phe Leu Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Thr Ala
 690 695 700
 15 Pro Asn Gln Ala Gln Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg
 705 710 715 720
 Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile
 725 730 735
 20 Trp Val Pro Glu Gly Glu Thr Val Lys Ile Pro Val Ala Ile Lys Ile
 740 745 750
 Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu
 755 760 765
 25 Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu
 770 775 780
 Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro
 785 790 795 800
 30 His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly
 805 810 815
 Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Met
 820 825 830
 35 Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn
 835 840 845
 Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu
 850 855 860
 Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly
 865 870 875 880
 45 Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys
 885 890 895
 Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu
 900 905 910
 50 Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu
 915 920 925

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Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
 930 935 940
 Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
 5 945 950 955 960
 Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
 965 970 975
 Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
 10 980 985 990
 Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
 995 1000 1005
 Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
 15 1010 1015 1020
 Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
 1025 1030 1035 1040
 Ile Asp Ser Asn Arg Ser Val Arg Asn Asn Tyr Ile His Ile Ser Tyr
 20 1045 1050 1055
 Ser Phe

(2) INFORMATION FOR SEQ ID NO:5:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

30 (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 156..1782

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CATTAGCTGC AATTGATCAA GTGACTGAGA GAAGGGCAAC ATTCCATGCA ACAGTATAGT 60
 40 GGTATGGAAA GCCCTGGATG TTGAAATCTA GCTTCAAAAA GCCTGTCTGG AAATGTAGTT 120
 AATTGGATGA AGTGAGAAGA GATAAAACCA GAGAG GAA GCT CTG ATC ATG GCA 173
 Glu Ala Leu Ile Met Ala
 1 5
 45 AGT ATG GAT CAT CCA CAC CTA GTC CGG TTG CTG GGT GTG TGT CTG AGC 221
 Ser Met Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser
 10 15 20

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	CCA ACC ATC CAG CTG GTT ACT CAA CTT ATG CCC CAT GGC TGC CTG TTG	269
	Pro Thr Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu	
	25 30 35	
5	GAG TAT GTC CAC GAG CAC AAG GAT AAC ATT GGA TCA CAA CTG CTG CTT	317
	Glu Tyr Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu	
	40 45 50	
	AAC TGG TGT GTC CAG ATA GCT AAG GGA ATG ATG TAC CTG GAA GAA AGA	365
	Asn Trp Cys Val Gln Ile Ala Lys Gly Met Tyr Leu Glu Glu Arg	
10	55 60 65 70	
	CGA CTC GTT CAT CGG GAT TTG GCA GCC CGT AAT GTC TTA GTG AAA TCT	413
	Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser	
	75 80 85	
15	CCA AAC CAT GTG AAA ATC ACA GAT TTT GGG CTA GCC AGA CTC TTG GAA	461
	Pro Asn His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu	
	90 95 100	
	GGA GAT GAA AAA GAG TAC AAT GCT GAT GGA GGA AAG ATG CCA ATT AAA	509
	Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys	
20	105 110 115	
	TGG ATG GCT CTG GAG TGT ATA CAT TAC AGG AAA TTC ACC CAT CAG AGT	557
	Trp Met Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser	
	120 125 130	
25	GAC GTT TGG AGC TAT GGA GTT ACT ATA TGG GAA CTG ATG ACC TTT GGA	605
	Asp Val Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly	
	135 140 145 150	
	GGA AAA CCC TAT GAT GGA ATT CCA ACG CGA GAA ATC CCT GAT TTA TTA	653
	Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu	
30	155 160 165	
	GAG AAA GGA GAA CGT TTG CCT CAG CCT CCC ATC TGC ACT ATT GAC GTT	701
	Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val	
	170 175 180	
35	TAC ATG GTC ATG GTC AAA TGT TGG ATG ATT GAT GCT GAC AGT AGA CCT	749
	Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro	
	185 190 195	
	AAA TTT AAG GAA CTG GCT GCT GAG TTT TCA AGG ATG GCT CGA GAC CCT	797
	Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro	
40	200 205 210	
	CAA AGA TAC CTA GTT ATT CAG GGT GAT GAT CGT ATG AAG CTT CCC AGT	845
	Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser	
	215 220 225 230	
45	CCA AAT GAC AGC AAG TTC TTT CAG AAT CTC TTG GAT GAA GAG GAT TTG	893
	Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu	
	235 240 245	
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	GAA	GAT	ATG	ATG	GAT	GCT	GAG	GAG	TAC	TTG	GTC	CCT	CAG	GCT	TTC	AAC	941
	Glu	Asp	Met	Met	Asp	Ala	Glu	Glu	Tyr	Leu	Val	Pro	Gln	Ala	Phe	Asn	
				250					255					260			
5	ATC	CCA	CCT	CCC	ATC	TAT	ACT	TCC	AGA	GCA	AGA	ATT	GAC	TCG	AAT	AGG	989
	Ile	Pro	Pro	Pro	Ile	Tyr	Thr	Ser	Arg	Ala	Arg	Ile	Asp	Ser	Asn	Arg	
			265					270					275				
	AGT	GAA	ATT	GGA	CAC	AGC	CCT	CCT	CCT	GCC	TAC	ACC	CCC	ATG	TCA	GGA	1037
10	Ser	Glu	Ile	Gly	His	Ser	Pro	Pro	Pro	Ala	Tyr	Thr	Pro	Met	Ser	Gly	
		280					285					290					
	AAC	CAG	TTT	GTA	TAC	CGA	GAT	GGA	GGT	TTT	GCT	GCT	GAA	CAA	GGA	GTG	1085
	Asn	Gln	Phe	Val	Tyr	Arg	Asp	Gly	Gly	Phe	Ala	Ala	Glu	Gln	Gly	Val	
		295				300				305						310	
15	TCT	GTG	CCC	TAC	AGA	GCC	CCA	ACT	AGC	ACA	ATT	CCA	GAA	GCT	CCT	GTG	1133
	Ser	Val	Pro	Tyr	Arg	Ala	Pro	Thr	Ser	Thr	Ile	Pro	Glu	Ala	Pro	Val	
				315						320					325		
	GCA	CAG	GGT	GCT	ACT	GCT	GAG	ATT	TTT	GAT	GAC	TCC	TGC	TGT	AAT	GGC	1181
20	Ala	Gln	Gly	Ala	Thr	Ala	Glu	Ile	Phe	Asp	Asp	Ser	Cys	Cys	Asn	Gly	
			330					335						340			
	ACC	CTA	CGC	AAG	CCA	GTG	GCA	CCC	CAT	GTC	CAA	GAG	GAC	AGT	AGC	ACC	1229
	Thr	Leu	Arg	Lys	Pro	Val	Ala	Pro	His	Val	Gln	Glu	Asp	Ser	Ser	Thr	
			345					350					355				
25	CAG	AGG	TAC	AGT	GCT	GAC	CCC	ACC	GTG	TTT	GCC	CCA	GAA	CGG	AGC	CCA	1277
	Gln	Arg	Tyr	Ser	Ala	Asp	Pro	Thr	Val	Phe	Ala	Pro	Glu	Arg	Ser	Pro	
		360				365						370					
	CGA	GGA	GAG	CTG	GAT	GAG	GAA	GGT	TAC	ATG	ACT	CCT	ATG	CGA	GAC	AAA	1325
30	Arg	Gly	Glu	Leu	Asp	Glu	Glu	Gly	Tyr	Met	Thr	Pro	Met	Arg	Asp	Lys	
		375			380				385							390	
	CCC	AAA	CAA	GAA	TAC	CTG	AAT	CCA	GTG	GAG	GAG	AAC	CCT	TTT	GTT	TCT	1373
	Pro	Lys	Gln	Glu	Tyr	Leu	Asn	Pro	Val	Glu	Glu	Asn	Pro	Phe	Val	Ser	
				395					400					405			
35	CGG	AGA	AAA	AAT	GGA	GAC	CTT	CAA	GCA	TTG	GAT	AAT	CCC	GAA	TAT	CAC	1421
	Arg	Arg	Lys	Asn	Gly	Asp	Leu	Gln	Ala	Leu	Asp	Asn	Pro	Glu	Tyr	His	
			410					415						420			
	AAT	GCA	TCC	AAT	GGT	CCA	CCC	AAG	GCC	GAG	GAT	GAG	TAT	GTG	AAT	GAG	1469
40	Asn	Ala	Ser	Asn	Gly	Pro	Pro	Lys	Ala	Glu	Asp	Glu	Tyr	Val	Asn	Glu	
			425					430					435				
	CCA	CTG	TAC	CTC	AAC	ACC	TTT	GCC	AAC	ACC	TTG	GGA	AAA	GCT	GAG	TAC	1517
	Pro	Leu	Tyr	Leu	Asn	Thr	Phe	Ala	Asn	Thr	Leu	Gly	Lys	Ala	Glu	Tyr	
		440				445					450						
45	CTG	AAG	AAC	AAC	ATA	CTG	TCA	ATG	CCA	GAG	AAG	GCC	AAG	AAA	GCG	TTT	1565
	Leu	Lys	Asn	Asn	Ile	Leu	Ser	Met	Pro	Glu	Lys	Ala	Lys	Lys	Ala	Phe	
		455			460					465						470	

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1 GAC AAC CCT GAC TAC TGG AAC CAC AGC CTG CCA CCT CGG AGC ACC CTT 1613
 Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu
 475 480 485
 5 CAG CAC CCA GAC TAC CTG CAG GAG TAC AGC ACA AAA TAT TTT TAT AAA 1661
 Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys
 490 495 500
 CAG AAT GGG CGG ATC CGG CCT ATT GTG GCA GAG AAT CCT GAA TAC CTC 1709
 Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu
 505 510 515
 10 TCT GAG TTC TCC CTG AAG CCA GGC ACT GTG CTG CCG CCT CCA CCT TAC 1757
 Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr
 520 525 530
 15 AGA CAC CGG AAT ACT GTG GTG TAAGCTCAGT TGTGGTTTTT TAGGTGGAGA 1808
 Arg His Arg Asn Thr Val Val
 535 540
 GACACACCTG CTCCAATTTC CCCACCCCCC TCTCTTTCTC TGGTGGTCTT CCTTCTACCC 1868
 20 CAAGGCCAGT AGTTTGTACA CTTCCCACTG GAAGATACAG AGATGCAATG ATAGTTATGT 1928
 GCTTACCTAA CTGGAACATT AGAGGGAAAG ACTGAAAGAG AAAGATAGGA GGAACCACAA 1988
 TGTTCCTTCA TTTCTCTGCA TGGGTTGGTC AGGAGAATGA AACAGCTAGA GAAGGACCAG 2048
 25 AAAATGTAAG GCAATGCTGC CTACTATCAA ACTAGCTGTC ACTTTTTTTC TTTTCTTTT 2108
 TCTTTCTTTG TTTCTTTCTT CCTCTTCTTT TTTTTTTTTT TTTTAAAGCA GATGGTTGAA 2168
 ACACCCATGC TATCTGTTCC TATCTGCAGG AACTGATGTG TGCATATTTA GCATCCCTGG 2228
 30 AAATCATAAT AAAGTTTCCA TTAGAACAAA AGAATAACAT TTTCTATAAC ATATGATAGT 2288
 GTCTGAAATT GAGAATCCAG TTTCTTTCCC CAGCAGTTTC TGTCTAGCA AGTAAGAATG 2348
 GCCAACTCAA CTTTCATAAT TTAAAAATCT CCATTAAAGT TATAACTAGT AATTATGTTT 2408
 35 TCAACACTTT TTGGTTTTTT TCATTTTGTT TTGCTCTGAC CGATTCCCTT ATATTTGCTC 2468
 CCCTATTTTT GGCTTTAATT TCTAATTGCA AAGATGTTTA CATCAAAGCT TCTTCACAGA 2528
 ATTTAAGCAA GAAATATTTT AATATAGTGA AATGGCCACT ACTTTAAGTA TACAATCTTT 2588
 40 AAAATAAGAA AGGGAGGCTA ATATTTTTC TACTATCAAA TTATCTTCAC CCTCATCCTT 2648
 TACATTTTTC AACATTTTTT TTTCTCCATA AATGACACTA CTTGATAGGC CGTTGGTTGT 2708
 CTGAAGAGTA GAAGGGAAAC TAAGAGACAG TTCTCTGTGG TTCAGGAAAA CTAAGTATAC 2768
 45 TTTCAGGGGT GGCCCAATGA GGGAAATCCAT TGAAGTGGAA GAAACACACT GGATTGGGTA 2828
 TGTCTACCTG GCAGATACTC AGAAATGTAG TTTGCACCTA AGCTGTAATT TTATTTGTTT 2888

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TTTTCTGAA CTCCATTTTG GATTTTGAAT CAAGCAATAT GGAAGCAACC AGCAAATTAA 2948
 CTAATTTAAG TACATTTTAA AAAAAGAGC TAAGATAAAG ACTGTGGAAA TGCCAAACCA 3008
 5 AGCAAATTAG GAACCTTGCA ACGGTATCCA GGGACTATGA TGAGAGGCCA GCACATTATC 3068
 TTCATATGTC ACCTTTGCTA CGCAAGGAAA TTTGTTCACT TCGTATACTT CGTAAGAAGG 3128
 AATGCGAGTA AGGATTGGCT TGAATTCCAT GGAATTTCTA GTATGAGACT ATTTATATGA 3188
 10 AGTAGAAGGT AACTCTTTGC ACATAAATTG GTATAATMAA AAGAAAAACA CAAACATTCA 3248
 AAGCTTAGGG ATAGGTCCTT GGGTCAAAAG TTGTAAATAA ATGTGAAACA TCTTCTCAAA 3308
 AAAAAAAAAA AAA 3321

15 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 541 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

25 Glu Ala Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu
 1 5 10 15
 Leu Gly Val Cys Leu Ser Pro Thr Ile Gln Leu Val Thr Gln Leu Met
 20 25 30
 30 Pro His Gly Cys Leu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile
 35 40 45
 Gly Ser Gln Leu Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met
 50 55 60
 35 Met Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg
 65 70 75 80
 Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly
 85 90 95
 40 Leu Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly
 100 105 110
 Gly Lys Met Pro Ile Lys Trp Met Ala Leu Glu Cys Ile His Tyr Arg
 115 120 125
 45 Lys Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Ile Trp
 130 135 140
 Glu Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg
 145 150 155 160

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Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pr Gln Pro Pro
 165 170 175
 5 Ile Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile
 180 185 190
 Asp Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser
 195 200 205
 10 Arg Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp
 210 215 220
 Arg Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu
 225 230 235 240
 15 Leu Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu
 245 250 255
 Val Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala
 260 265 270
 20 Arg Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala
 275 280 285
 Tyr Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe
 290 295 300
 25 Ala Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr
 305 310 315 320
 Ile Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp
 325 330 335
 30 Asp Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val
 340 345 350
 Gln Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe
 355 360 365
 35 Ala Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met
 370 375 380
 Thr Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu
 385 390 395 400
 Glu Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu
 405 410 415
 45 Asp Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu
 420 425 430
 Asp Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr
 435 440 445
 50 Leu Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu
 450 455 460

55

Lys Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu
465 470 475 480

Pro Pr Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser
485 490 495

Thr Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala
500 505 510

Glu Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val
515 520 525

Leu Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
530 535 540

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1210 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
1 5 10 15

Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
20 25 30

Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
35 40 45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
50 55 60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65 70 75 80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
85 90 95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
100 105 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
115 120 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
130 135 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
 145 150 155 160
 5 Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
 165 170 175
 Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
 180 185 190
 10 Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
 195 200 205
 Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
 210 215 220
 15 Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
 225 230 235 240
 Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
 245 250 255
 20 Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
 260 265 270
 Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
 275 280 285
 25 Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
 290 295 300
 Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
 305 310 315 320
 30 Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
 325 330 335
 Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
 340 345 350
 35 Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
 355 360 365
 Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
 370 375 380
 40 Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
 385 390 395 400
 Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
 405 410 415
 45 Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
 420 425 430
 50 His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
 435 440 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
 450 455 460
 6 Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
 465 470 475 480
 Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
 485 490 495
 10 Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
 500 505 510
 Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
 515 520 525
 16 Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Lys Leu Leu Glu Gly
 530 535 540
 Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
 545 550 555 560
 20 Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
 565 570 575
 Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
 580 585 590
 26 Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
 595 600 605
 Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
 610 615 620
 30 Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
 625 630 635 640
 Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
 645 650 655
 35 Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
 660 665 670
 Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
 675 680 685
 Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
 690 695 700
 45 Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
 705 710 715 720
 Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
 725 730 735
 50 Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
 740 745 750

55

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
 755 760 765
 5 Val Asp Asn Pr His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
 770 775 780
 Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
 785 790 795 800
 10 Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
 805 810 815
 Trp Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Asp Arg Arg
 820 825 830
 15 Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
 835 840 845
 Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
 850 855 860
 20 Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
 865 870 875 880
 Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
 885 890 895
 25 Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
 900 905 910
 Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
 915 920 925
 30 Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
 930 935 940
 Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
 945 950 955 960
 35 Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
 965 970 975
 Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
 980 985 990
 40 Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
 995 1000 1005
 Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
 1010 1015 1020
 45 Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
 1025 1030 1035 1040
 50 Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
 1045 1050 1055

55

Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser S r Asp
 1060 1065 1070
 5 Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
 1075 1080 1085
 Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
 1090 1095 1100
 10 Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
 1105 1110 1115 1120
 Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
 1125 1130 1135
 15 Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
 1140 1145 1150
 Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
 1155 1160 1165
 20 Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
 1170 1175 1180
 Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
 1185 1190 1195 1200
 25 Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
 1205 1210

(2) INFORMATION FOR SEQ ID NO:8:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1255 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

40 Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1 5 10 15
 Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
 20 25 30
 45 Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
 35 40 45
 Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
 50 55 60

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Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
 65 70 75 80
 5 Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pr Leu
 85 90 95
 Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
 100 105 110
 10 Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
 115 120 125
 Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
 130 135 140
 15 Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
 145 150 155 160
 Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
 165 170 175
 20 Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
 180 185 190
 His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
 195 200 205
 25 Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
 210 215 220
 Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
 225 230 235 240
 30 Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
 245 250 255
 His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
 260 265 270
 35 Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
 275 280 285
 Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
 290 295 300
 40 Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
 305 310 315 320
 45 Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
 325 330 335
 Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
 340 345 350
 50 Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
 355 360 365

55

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pr Glu Ser Phe Asp Gly Asp
 370 375 380
 Pro Ala Ser Asn Thr Ala Pro Leu Gln Pr Glu Gln Leu Gln Val Phe
 8 385 390 395 400
 Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
 405 410 415
 Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
 10 420 425 430
 Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
 435 440 445
 Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
 15 450 455 460
 Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
 465 470 475 480
 Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
 20 485 490 495
 Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
 500 505 510
 Gln Leu Cys Ala Arg Arg Ala Leu Leu Gly Ser Gly Pro Thr Gln Cys
 25 515 520 525
 Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
 530 535 540
 Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
 30 545 550 555 560
 Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
 565 570 575
 Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
 35 580 585 590
 Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
 595 600 605
 Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
 40 610 615 620
 Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
 45 625 630 635 640
 Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Val Ser
 645 650 655
 Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
 50 660 665 670

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Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
 675 680 685
 5 Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pr Leu Thr Pr Ser Gly
 690 695 700
 Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
 705 710 715 720
 10 Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
 725 730 735
 Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
 740 745 750
 15 Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
 755 760 765
 Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
 770 775 780
 20 Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
 785 790 795 800
 Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
 805 810 815
 25 Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
 820 825 830
 Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
 835 840 845
 30 Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
 850 855 860
 Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
 865 870 875 880
 35 Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
 885 890 895
 Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
 900 905 910
 40 Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
 915 920 925
 Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
 930 935 940
 45 Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
 945 950 955 960
 Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
 965 970 975
 50
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Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
 980 985 990
 8 Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
 995 1000 1005
 Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
 1010 1015 1020
 10 Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
 1025 1030 1035 1040
 Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
 1045 1050 1055
 15 Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
 1060 1065 1070
 Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
 1075 1080 1085
 20 Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
 1090 1095 1100
 Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
 1105 1110 1115 1120
 25 Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
 1125 1130 1135
 Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
 1140 1145 1150
 30 Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
 1155 1160 1165
 Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
 1170 1175 1180
 35 Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
 1185 1190 1195 1200
 Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
 1205 1210 1215
 40 Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
 1220 1225 1230
 Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Val Ala Glu Asn Pro Glu
 1235 1240 1245
 45 Tyr Gly Leu Asp Val Pro Val
 1250 1255

(2) INFORMATION FOR SEQ ID NO:9:

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(i) SEQUENCE CHARACTERISTICS:

55

(A) LENGTH: 1342 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
1           5           10           15
Ala Arg Gly Ser Glu Val Gly Asn Ser Gln Ala Val Cys Pro Gly Thr
15          20          25          30
Leu Asn Gly Leu Ser Val Thr Gly Asp Ala Glu Asn Gln Tyr Gln Thr
35          40          45
Leu Tyr Lys Leu Tyr Glu Arg Cys Glu Val Val Met Gly Asn Leu Glu
20          50          55          60
Ile Val Leu Thr Gly His Asn Ala Asp Leu Ser Phe Leu Gln Trp Ile
65          70          75          80
Arg Glu Val Thr Gly Tyr Val Leu Val Ala Met Asn Glu Phe Ser Thr
25          85          90          95
Leu Pro Leu Pro Asn Leu Arg Val Val Arg Gly Thr Gln Val Tyr Asp
30          100         105         110
Gly Lys Phe Ala Ile Phe Val Met Leu Asn Tyr Asn Thr Asn Ser Ser
115         120         125
His Ala Leu Arg Gln Leu Arg Leu Thr Gln Leu Thr Glu Ile Leu Ser
35          130         135         140
Gly Gly Val Tyr Ile Glu Lys Asn Asp Lys Leu Cys His Met Asp Thr
145         150         155         160
Ile Asp Trp Arg Asp Ile Val Arg Asp Arg Asp Ala Glu Ile Val Val
40          165         170         175
Lys Asp Asn Gly Arg Ser Cys Pro Pro Cys His Glu Val Cys Lys Gly
180         185         190
Arg Cys Trp Gly Pro Gly Ser Glu Asp Cys Gln Thr Leu Thr Lys Thr
45          195         200         205
Ile Cys Ala Pro Gln Cys Asn Gly His Cys Phe Gly Pro Asn Pro Asn
210         215         220
Gln Cys Cys His Asp Glu Cys Ala Gly Gly Cys Ser Gly Pro Gln Asp
50          225         230         235         240

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Thr Asp Cys Phe Ala Cys Arg His Phe Asn Asp Ser Gly Ala Cys Val
 245 250 255
 8 Pro Arg Cys Pro Gln Pro Leu Val Tyr Asn Lys Leu Thr Phe Gln Leu
 260 265 270
 Glu Pro Asn Pro His Thr Lys Tyr Gln Tyr Gly Gly Val Cys Val Ala
 275 280 285
 10 Ser Cys Pro His Asn Phe Val Val Asp Gln Thr Ser Cys Val Arg Ala
 290 295 300
 Cys Pro Pro Asp Lys Met Glu Val Asp Lys Asn Gly Leu Lys Met Cys
 305 310 315 320
 15 Glu Pro Cys Gly Gly Leu Cys Pro Lys Ala Cys Glu Gly Thr Gly Ser
 325 330 335
 Gly Ser Arg Phe Gln Thr Val Asp Ser Ser Asn Ile Asp Gly Phe Val
 340 345 350
 20 Asn Cys Thr Lys Ile Leu Gly Asn Leu Asp Phe Leu Ile Thr Gly Leu
 355 360 365
 Asn Gly Asp Pro Trp His Lys Ile Pro Ala Leu Asp Pro Glu Lys Leu
 370 375 380
 25 Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly Tyr Leu Asn Ile Gln
 385 390 395 400
 Ser Trp Pro Pro His Met His Asn Phe Ser Val Phe Ser Asn Leu Thr
 405 410 415
 30 Thr Ile Gly Gly Arg Ser Leu Tyr Asn Arg Gly Phe Ser Leu Leu Ile
 420 425 430
 Met Lys Asn Leu Asn Val Thr Ser Leu Gly Phe Arg Ser Leu Lys Glu
 435 440 445
 35 Ile Ser Ala Gly Arg Ile Tyr Ile Ser Ala Asn Arg Gln Leu Cys Tyr
 450 455 460
 His His Ser Leu Asn Trp Thr Lys Val Leu Arg Gly Pro Thr Glu Glu
 465 470 475 480
 Arg Leu Asp Ile Lys His Asn Arg Pro Arg Arg Asp Cys Val Ala Glu
 485 490 495
 45 Gly Lys Val Cys Asp Pro Leu Cys Ser Ser Gly Gly Cys Trp Gly Pro
 500 505 510
 Gly Pro Gly Gln Cys Leu Ser Cys Arg Asn Tyr Ser Arg Gly Gly Val
 515 520 525
 50 Cys Val Thr His Cys Asn Phe Leu Asn Gly Glu Pro Arg Glu Phe Ala
 530 535 540

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His Glu Ala Glu Cys Phe Ser Cys His Pro Glu Cys Gln Pro Met Gly
 545 550 555 560
 5 Gly Thr Ala Thr Cys Asn Gly Ser Gly Ser Asp Thr Cys Ala Gln Cys
 565 570 575
 Ala His Phe Arg Asp Gly Pro His Cys Val Ser Ser Cys Pro His Gly
 580 585 590
 10 Val Leu Gly Ala Lys Gly Pro Ile Tyr Lys Tyr Pro Asp Val Gln Asn
 595 600 605
 Glu Cys Arg Pro Cys His Glu Asn Cys Thr Gln Gly Cys Lys Gly Pro
 610 615 620
 15 Glu Leu Gln Asp Cys Leu Gly Gln Thr Leu Val Leu Ile Gly Lys Thr
 625 630 635 640
 His Leu Thr Met Ala Leu Thr Val Ile Ala Gly Leu Val Val Ile Phe
 645 650 655
 20 Met Met Leu Gly Gly Thr Phe Leu Tyr Trp Arg Gly Arg Arg Ile Gln
 660 665 670
 Asn Lys Arg Ala Met Arg Arg Tyr Leu Glu Arg Gly Glu Ser Ile Glu
 675 680 685
 25 Pro Leu Asp Pro Ser Glu Lys Ala Asn Lys Val Leu Ala Arg Ile Phe
 690 695 700
 Lys Glu Thr Glu Leu Arg Lys Leu Lys Val Leu Gly Ser Gly Val Phe
 705 710 715 720
 30 Gly Thr Val His Lys Gly Val Trp Ile Pro Glu Gly Glu Ser Ile Lys
 725 730 735
 Ile Pro Val Cys Ile Lys Val Ile Glu Asp Lys Ser Gly Arg Gln Ser
 740 745 750
 35 Phe Gln Ala Val Thr Asp His Met Leu Ala Ile Gly Ser Leu Asp His
 755 760 765
 40 Ala His Ile Val Arg Leu Leu Gly Leu Cys Pro Gly Ser Ser Leu Gln
 770 775 780
 Leu Val Thr Gln Tyr Leu Pro Leu Gly Ser Leu Leu Asp His Val Arg
 785 790 795 800
 45 Gln His Arg Gly Ala Leu Gly Pro Gln Leu Leu Asn Trp Gly Val
 805 810 815
 Gln Ile Ala Lys Gly Met Tyr Tyr Leu Glu Glu His Gly Met Val His
 820 825 830
 50 Arg Asn Leu Ala Ala Arg Asn Val Leu Leu Lys Ser Pro Ser Gln Val
 835 840 845

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Gln Val Ala Asp Phe Gly Val Ala Asp Leu Leu Pro Pro Asp Asp Lys
 850 855 860
 6 Gln Leu Leu Tyr Ser Glu Ala Lys Thr Pro Ile Lys Trp Met Ala Leu
 865 870 875 880
 Glu Ser Ile His Phe Gly Lys Tyr Thr His Gln Ser Asp Val Trp Ser
 885 890 895
 10 Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ala Glu Pro Tyr
 900 905 910
 Ala Gly Leu Arg Leu Ala Glu Val Pro Asp Leu Leu Glu Lys Gly Glu
 915 920 925
 15 Arg Leu Ala Gln Pro Gln Ile Cys Thr Ile Asp Val Tyr Met Val Met
 930 935 940
 Val Lys Cys Trp Met Ile Asp Glu Asn Ile Arg Pro Thr Phe Lys Glu
 945 950 955 960
 20 Leu Ala Asn Glu Phe Thr Arg Met Ala Arg Asp Pro Pro Arg Tyr Leu
 965 970 975
 Val Ile Lys Arg Glu Ser Gly Pro Gly Ile Ala Pro Gly Pro Glu Pro
 980 985 990
 25 His Gly Leu Thr Asn Lys Lys Leu Glu Glu Val Glu Leu Glu Pro Glu
 995 1000 1005
 Leu Asp Leu Asp Leu Asp Leu Glu Ala Glu Glu Asp Asn Leu Ala Thr
 1010 1015 1020
 30 Thr Thr Leu Gly Ser Ala Leu Ser Leu Pro Val Gly Thr Leu Asn Arg
 1025 1030 1035 1040
 Pro Arg Gly Ser Gln Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro
 1045 1050 1055
 35 Met Asn Gln Gly Asn Leu Gly Gly Ser Cys Gln Glu Ser Ala Val Ser
 1060 1065 1070
 Gly Ser Ser Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro
 1075 1080 1085
 40 Arg Gly Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser
 1090 1095 1100
 Glu Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
 1105 1110 1115 1120
 Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln Arg
 1125 1130 1135
 50 His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly Leu Glu
 1140 1145 1150

55

Glu Glu Asp Val Asn Gly Tyr Val Met Pr Asp Thr His Leu Lys Gly
 1155 1160 1165
 Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val Gly Leu Ser Ser
 1170 1175 1180
 Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu Glu Tyr Glu Tyr Met
 1185 1190 1195 1200
 Asn Arg Arg Arg Arg His Ser Pro Pro His Pro Pro Arg Pro Ser Ser
 1205 1210 1215
 Leu Glu Glu Leu Gly Tyr Glu Tyr Met Asp Val Gly Ser Asp Leu Ser
 1220 1225 1230
 Ala Ser Leu Gly Ser Thr Gln Ser Cys Pro Leu His Pro Val Pro Ile
 1235 1240 1245
 Met Pro Thr Ala Gly Thr Thr Pro Asp Glu Asp Tyr Glu Tyr Met Asn
 1250 1255 1260
 Arg Gln Arg Asp Gly Gly Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly
 1265 1270 1275 1280
 Ala Cys Pro Ala Ser Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln
 1285 1290 1295
 Gly Pro Gly His Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr
 1300 1305 1310
 Leu Arg Ser Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr
 1315 1320 1325
 Trp His Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
 1330 1335 1340

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 911 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1 5 10 15
 Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
 20 25 30

Glu Asn Lys Leu Ser S r Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
 35 40 45
 5 Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
 50 55 60
 Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
 65 70 75 80
 10 Arg Glu Val Thr Gly Tyr Val Leu Val Ala Leu Asn Gln Phe Arg Tyr
 85 90 95
 Leu Pro Leu Glu Asn Leu Arg Ile Ile Arg Gly Thr Lys Leu Tyr Glu
 100 105 110
 15 Asp Arg Tyr Ala Leu Ala Ile Phe Leu Asn Tyr Arg Lys Asp Gly Asn
 115 120 125
 Phe Gly Leu Gln Glu Leu Gly Leu Lys Asn Leu Thr Glu Ile Leu Asn
 130 135 140
 20 Gly Gly Val Tyr Val Asp Gln Asn Lys Phe Leu Cys Tyr Ala Asp Thr
 145 150 155 160
 Ile His Trp Gln Asp Ile Val Arg Asn Pro Trp Pro Ser Asn Leu Thr
 165 170 175
 25 Leu Val Ser Thr Asn Gly Ser Ser Gly Cys Gly Arg Cys His Lys Ser
 180 185 190
 Cys Thr Gly Arg Cys Trp Gly Pro Thr Glu Asn His Cys Gln Thr Leu
 195 200 205
 30 Thr Arg Thr Val Cys Ala Glu Gln Cys Asp Gly Arg Cys Tyr Gly Pro
 210 215 220
 Tyr Val Ser Asp Cys Cys His Arg Glu Cys Ala Gly Gly Cys Ser Gly
 225 230 235 240
 35 Pro Lys Asp Thr Asp Cys Phe Ala Cys Met Asn Phe Asn Asp Ser Gly
 245 250 255
 Ala Cys Val Thr Gln Cys Pro Gln Thr Phe Val Tyr Asn Pro Thr Thr
 260 265 270
 40 Phe Gln Leu Glu His Asn Phe Asn Ala Lys Tyr Thr Tyr Gly Ala Phe
 275 280 285
 Cys Val Lys Lys Cys Pro His Asn Phe Val Val Asp Ser Ser Ser Cys
 290 295 300
 45 Val Arg Ala Cys Pro Ser Ser Lys Met Glu Val Glu Glu Asn Gly Ile
 305 310 315 320
 50 Lys Met Cys Lys Pro Cys Thr Asp Ile Cys Pro Lys Ala Cys Asp Gly
 325 330 335

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Ile Gly Thr Gly Ser Leu Met Ser Ala Gln Thr Val Asp Ser Ser Asn
 340 345 350
 5 Ile Asp Lys Ph Ile Asn Cys Thr Lys Ile Asn Gly Asn Leu Ile Phe
 355 360 365
 Leu Val Thr Gly Ile His Gly Asp Pr Tyr Asn Ala Ile Glu Ala Ile
 370 375 380
 10 Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly
 385 390 395 400
 Phe Leu Asn Ile Gln Ser Trp Pro Pro Asn Met Thr Asp Phe Ser Val
 405 410 415
 15 Phe Ser Asn Leu Val Thr Ile Gly Gly Arg Val Leu Tyr Ser Gly Leu
 420 425 430
 Ser Leu Leu Ile Leu Lys Gln Gln Gly Ile Thr Ser Leu Gln Phe Gln
 435 440 445
 20 Ser Leu Lys Glu Ile Ser Ala Gly Asn Ile Tyr Ile Thr Asp Asn Ser
 450 455 460
 Asn Leu Cys Tyr Tyr His Thr Ile Asn Trp Thr Thr Leu Phe Ser Thr
 465 470 475 480
 25 Ile Asn Gln Arg Ile Val Ile Arg Asp Asn Arg Lys Ala Glu Asn Cys
 485 490 495
 Thr Ala Glu Gly Met Val Cys Asn His Leu Cys Ser Ser Asp Gly Cys
 500 505 510
 30 Trp Gly Pro Gly Pro Asp Gln Cys Leu Ser Cys Arg Arg Phe Ser Arg
 515 520 525
 Gly Arg Ile Cys Ile Glu Ser Cys Asn Leu Tyr Asp Gly Glu Phe Arg
 530 535 540
 35 Glu Phe Glu Asn Gly Ser Ile Cys Val Glu Cys Asp Pro Gln Cys Glu
 545 550 555 560
 Lys Met Glu Asp Gly Leu Leu Thr Cys His Gly Pro Gly Pro Asp Asn
 565 570 575
 40 Cys Thr Lys Cys Ser His Phe Lys Asp Gly Pro Asn Cys Val Glu Lys
 580 585 590
 Cys Pro Asp Gly Leu Gln Gly Ala Asn Ser Phe Ile Phe Lys Tyr Ala
 595 600 605
 45 Asp Pro Asp Arg Glu Cys His Pro Cys His Pro Asn Cys Thr Gln Gly
 610 615 620
 Cys Asn Gly Pro Thr Ser His Asp Cys Ile Tyr Tyr Pro Trp Thr Gly
 625 630 635 640
 50
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His Ser Thr Leu Pro Gln Asp Pro Val Lys Val Lys Ala Leu Glu Gly
 645 650 655
 6 Phe Pro Arg Leu Val Gly Pro Asp Phe Phe Gly Cys Ala Glu Pro Ala
 660 665 670
 Asn Thr Phe Leu Asp Pro Glu Glu Pro Lys Ser Cys Asp Lys Thr His
 675 680 685
 10 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
 690 695 700
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 705 710 715 720
 15 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 725 730 735
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Val Ala Lys
 740 745 750
 20 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 755 760 765
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 770 775 780
 25 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 785 790 795 800
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 805 810 815
 30 Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 820 825 830
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 835 840 845
 35 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 850 855 860
 Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 865 870 875 880
 40 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 885 890 895
 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 900 905 910
 45

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Xaa Gly Xaa Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Leu Ala Ala Arg Asn
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Ile Lys Trp Met Ala
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:14:
ACNGTNTGGG ARYTNAYHAC 20

6 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

15 (x1) SEQUENCE DESCRIPTION: SEQ ID NO:15:
CAYGTNAARA THACNGAYTT YGG 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic) 25

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:16:
30 GACGAATTCC NATHAARTGG ATGGC 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

40 (x1) SEQUENCE DESCRIPTION: SEQ ID NO:17:
ACAYTTNARD ATDATCATRT ANAC 24

(2) INFORMATION FOR SEQ ID NO:18:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AANGTCATNA RYTCCCA

17

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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

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(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCCAGNGCGA TCCAYTTDAT NGG

23

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(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

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(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGRTCDATCA TCCARCCT

18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

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(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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CTGCTGTCAG CATCGATCAT

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(2) INFORMATION FOR SEQ ID NO:22:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

- 10 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

15 Thr Val Trp Glu Leu Met Thr
1 5

(2) INFORMATION FOR SEQ ID NO:23:

- 20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

- 25 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

30 His Val Lys Ile Thr Asp Phe Gly
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

40 Val Tyr Met Ile Ile Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:25:

- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids

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(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Trp Glu Leu Met Thr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Pro Ile Lys Trp Met Ala Leu Glu
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Trp Met Ile Asp Pro
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GACTCGAGTC GACATCGATT TTTTTTTTTT TTTT

35

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAAGAAAGAC GACTCGTTCA TCGG

24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GACCATGACC ATGTAAACGT CAATA

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Claims

1. A recombinant polynucleotide comprising a sequence of at least about 200 nucleotides having greater than 80 % homology to a contiguous portion of the HER4 nucleotide sequence depicted in FIG. 1 or its complement.
2. The recombinant polynucleotide of claim 1 comprising a sequence of nucleotides encoding at least about 70 contiguous amino acids within the HER4 amino acid sequence depicted in FIG. 1.
3. The recombinant polynucleotide of claim 1 comprising a contiguous sequence of at least about 200 nucleotides within the HER4 nucleotide coding sequence depicted in FIG. 1 or its complement.
4. The recombinant polynucleotide of claim 1 comprising the HER4 nucleotide coding sequence depicted in FIG. 1 or its complement.
5. A recombinant polynucleotide which encodes a polypeptide having structural characteristics equivalent to that of HER4, which polynucleotide is obtained by single or multiple base addition, deletion and/or substitution in a nucleotide sequence of one of the claims 1 to 4, or which is obtained by selective hybridization with a nucleotide sequence of one of the claims 1 to 4.
6. A recombinant polynucleotide according to one of the claims 1 to 5 which is a DNA polynucleotide.
7. A recombinant polynucleotide according to one of the claims 1 to 5 which is a RNA polynucleotide.

8. An assay kit comprising a recombinant polynucleotide according to one of the claims 1 to 5 to which a detectable label has been added.
9. A polymerase chain reaction (PCR) kit comprising a pair of primers capable of priming cDNA synthesis in a PCR reaction, wherein each primer is a polynucleotide according to claim 6.
10. The PCR kit according to claim 9 further comprising a polynucleotide probe capable of hybridizing to a region of the HER4 gene between and not including the nucleotide sequences to which the primers hybridize.
11. A polypeptide comprising a sequence of at least about 80 amino acids having greater than 90 % identity to a contiguous portion of the HER4 amino acid sequence depicted in FIG. 1.
12. A HER4 polypeptide comprising
 - the amino acid sequence depicted in FIG. 1 from amino acid residues 1 through 1308; or
 - the amino acid sequence depicted in FIG. 1 from amino acid residues 26 through 1308; or
 - the amino acid sequence depicted in FIG. 1 from amino acid residues 1 through 1045; or
 - the amino acid sequence depicted in FIG. 1 from amino acid residues 26 through 1045; or
 - the amino acid sequence depicted in FIG. 2A, or
 - the amino acid sequence depicted in FIG. 1 from amino acid residues 772 through 1308; or
 - the amino acid sequence depicted in FIG. 2B.
13. A polypeptide having structural and/or functional features equivalent to HER4, obtainable by single or multiple amino acid addition, deletion and/or substitution in a sequence of one of the claims 11 or 12.
14. An antibody capable of inhibiting the interaction of a soluble polypeptide and human HER4.
15. An antibody according to claim 14 wherein the soluble polypeptide is a heregulin.
16. An antibody capable of
 - a) stimulating HER4 tyrosine autophosphorylation; or
 - b) inducing a HER4-mediated signal in a cell, which signal results in modulation of growth and/or differentiation of the cell; or
 - c) inhibiting HepG2 fraction 17-stimulated tyrosine phosphorylation of HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC (accession number CRL 11205).
17. An antibody which immunospecifically binds to human HER4.
18. An antibody according to claim 17 which
 - a) resides on the cell surface after binding to HER4; or
 - b) is internalized into the cell after binding to HER4; or
 - c) immunospecifically binds to human HER4 expressed in CHO/HER4 21-2 cells as deposited with the ATCC (accession number CRL 11205); or
 - d) neutralizes HER4 biological activity; or
 - e) is conjugated to a drug or toxin; or
 - f) is radiolabeled.
19. Plasmid pBSHER4Y as deposited with the ATCC and having the accession number ATCC 89131.
20. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to one of the claims 11 to 13.
21. A host cell transfected with a recombinant vector according to claim 20.
22. A recombinant vector comprising a nucleotide sequence encoding a polypeptide according to one of the claims 11 to 13 wherein the coding sequence is operably linked to a control sequence which is capable of directing the expression of the coding sequence in a host cell transfected therewith.

23. A host cell transfected with a recombinant vector according to claim 22.
24. Cell line CHO/HER4 21-2 as deposited with the ATCC and having the accession number CRL 11205.
- 6 25. An assay for detecting the presence of a HER4 ligand in a sample comprising:
(a) applying the sample to cells which have been engineered to overexpress HER4; and
(b) detecting an ability of the ligand to affect an activity mediated by HER4.
- 10 26. The method according to claim 25, wherein the cells are CHO/HER4 21-2 cells as deposited with the ATCC and having the accession number CRL 11205.
27. The method according to claim 25, wherein the activity detected is HER4 tyrosine phosphorylation, or morphologic differentiation.
- 15 28. A ligand for HER4 comprising a polypeptide which binds to HER4, stimulates tyrosine phosphorylation of HER4, and affects a biological activity mediated by HER4.
29. A ligand according to claim 28 which is capable of inducing morphological differentiation when added to cultured MDA-MB-453 cells; and/or which is obtained from cultured HepG2 cell conditioned media.
- 20 30. An immunoassay for detecting HER4 comprising:
a) providing an antibody according to claim 17 or 18;
b) incubating a biological sample with the antibody under conditions which allow for the binding of the antibody to HER4; and
25 c) determining the amount of antibody present as a HER4-antibody complex.
31. The use of at least one antibody according to one of the claims 17 or 18 for preparing a pharmaceutical composition for the in vivo delivery of a drug or toxin to cells expressing HER4.
- 30 32. The use of claim 31, which comprises conjugating at least one antibody according to claim 17 or 18, or an active fragment thereof, to the drug or toxin, for delivering the resulting conjugate to an individual by using a formulation, dose, and route of administration such that the conjugate binds to HER4.

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1	MetLysProAlaThrGlyLeuTrpValTrp	ValSerLeuLeuValAlaAlaGlyThr
1	AATGTGACGACGGGATCTGAGACTTCCAAAA	ATGAAGCCGGCAGACAGGACTTTGGGTCTGG
20	Val GlnProSerAspSerGlnSerValCysAla	GlyThrGluAsnLysLeuSerSerLeuSer
91	GTC CAGCCACGCGATTCTCAGTCAGTGTGCA	GGACGGAGAAATAAACTGAGCTCTCTCTCT
50	Arg LysTyrTyrGluAsnCysGluValValMet	GlyAsnLeuGluIleThrSerIleGluHis
181	CGC AAGTACTATGAAAACGTGAGGTTGTCA TG	GGCAACCTGGAGATAACCAAGCATTTGGCAC
80	Val ArgGluValThrGlyTyrValLeuValAla	LeuAsnGlnPheArgTyrLeuProLeuGlu
271	GTT CGAGAACTCACAGGCTACGTGTTAGTGGCT	CTTAATCAGTTTCGTACCTGCCTCTGGAG
110	Leu TyrGluAspArgTyrAlaLeuAlaIlePhe	LeuAsnTyrArgLysAspGlyAsnPheGly
361	CTT TATCAGGATCGATATGCTTGGCAATATT	TTAAACTACAGAAAAGATGGAACTTTGGA
140	Thr GluIleLeuAsnGlyGlyValTyrValAsp	GlnAsnLysPheLeuCysTyrAlaAspThr
451	ACA GAAATCTAAATGGTGGAGTCTATGTAGAC	CAGAACAAATTCCTTTGTTATGCAGACACC
170	Pro TrpProSerAsnLeuThrLeuValSerThr	AsnGlySerSerGlyCysGlyArgCysHis
541	CCA TGGCTTCCAACTTGACTCTTGCTCAACA	AATGGTAGTTCCAGGATGTGGACGTGCCAT
200	Pro ThrGluAsnHisCysGlnThrLeuThrArg	ThrValCysAlaGluGlnCysAspGlyArg
631	CCC ACAGAAATCATTCGCAGACTTTGCACANG	ACGGTGTGTCCAGAACAAATGTGACGGCAGA
230	Cys HisArgGluCysAlaGlyCysSerGly	ProLysAspThrAspCysPheAlaCysMet
721	TGC CATCGAGAAATGTGCTGGAGGCTGCTCAGGA	CCTAAGGACACAGACTGCTTTGCTGECATG
260	Thr GlnCysProGlnThrPheValTyrAsnPro	ThrThrPheGlnLeuGluHisAsnPheAsn
811	ACT CAGTGTCCCCAAACCTTTGTCTACAATCCA	ACCACCTTTTCACTGGAGCACAAATTTCAAT
290	Val LysLysCysProHisAsnPheValValAsp	SerSerSerCysValArgAlaCysProSer
901	GTC AAGAAATGTCCACATAACTTTGTGGTAGAT	TCCAGTTCTTGTGTGCGTGCCTGCCCTAGT
320	Ile LysMetCysLysProCysThrAspIleCys	ProLysAlaCysAspGlyIleGlyThrGly
991	ATT AAAATGTGTAACCTTGCACATGACATTTC	CCAAAAGCTTGTGATGGCATTTGCCACAGGA
		TCATTGATGTAGCTCAGACTGTGGAT

Figure 1

350 Ser SerAsnIleAspLysPheIleAsnCysThr LysIleAsnGlyAsnLeuIlePheLeuVal ThrGlyIleHisGlyAspPp TyrAsn
 1081 TCC AGTAACATTGACAAATTCATAAACTGTACC AAGATCAATGGGAATTTCATCTTTCTAGTC ACTGGTATTCAATGGGACCCCTTACAAAT
 380 Ala IleGluAlaIleAspProGluLysLeuAsn ValPheArgThrValArgGluIleThrGly PheLeuAsnIleGlnSerTppProPro
 1171 GCA ATTGAAGCCATAGACCCACAGAAACTGAAC GTCTTTCCGACAGTCACAGAGATAACAGGT TTCTGAACATACAGTCAATGGCCACCA
 410 Asn MetThrAspPheSerValPheSerAsnLeu ValThrIleGlyGlyArgValLeuTyrSer GlyLeuSerLeuLeuIleLeuLysGln
 1261 AAC ATGACTGACTTCAGTGTTTTTTCTAACCTG GTGACCAATGGTGGAGAGTACTCTATAGT GGCCTGTCTCTGCTTATCTCAAGCAA
 440 Gln GlyIleThrSerLeuGlnPheGlnSerLeu LysGluIleSerAlaGlyAsnIleTyrIle ThrAspAsnSerAsnLeuCysTyrTyr
 1351 CAG GGCATCACCTCTCTACAGTTCAGTCCCTG AAGGAATCAGCGCAGGAACATCTATATT ACTGACAAACAGCAACCTGTGTATTAT
 470 His ThrIleAsnTppThrThrLeuPheSerThr IleAsnGlnArgIleValIleArgAspAsn ArgLysAlaGluAsnCysThrAlaGlu
 1441 CAT ACCATTAACTGGACAAACACTCTTCAGCACA ATCAACAGAGAAATAGTAATCCGGGACAAAC AGAAAGCTGAAATTTGTACTGCTGAA
 500 Gly MetValCysAsnHisLeuCysSerSerAsp GlyCysTppGlyProGlyProAspGlnCys LeuSerCysArgArgPheSerArgGly
 1531 GGA ATGCTGTGCACCAACCATCTGTGTCCAGTGAT GGCTGTGGGACCTGGGCCGACCAATGT CTGTCTGTCTCCCTTCAGTAGAGCA
 530 Arg IleCysIleGluSerCysAsnLeuTyrAsp GlyGluPheArgGluPheGluAsnGlySer IleCysValGluCysAspProGlnCys
 1621 AGG ATCTGCATAGAGTCTTGTAACTCTATGAT GGTGAATTTCCGGAGTTTGAGAAATGGCTCC ATCTGTGTGGAGTGTGACCCCACTGT
 560 Glu LysMetGluAspGlyLeuLeuThrCysHis GlyProGlyProAspAsnCysThrLysCys SerHisPheLysAspGlyProAsnCys
 1711 GAG AAGATGGAAGATGGCTCTCCATGCCAT GGACCGGCTCTGACAACTGTACAAAGTGC TCTCATTTTAAAGATGGCCCAAACTGT
 590 Val GluLysCysProAspGlyLeuGlnGlyAla AsnSerPheIlePheLysTyrAlaAspPro AspArgGluCysHisProCysHisPro
 1801 GTG GAAATGTCCAGATGGCTTACAGGGGCA AACAGTTTCATTTTCAAGTATGCTGATCCA GATCGGAGTGCACCCATGCCATCCA
 620 Asn CysThrGlnGlyCysAsnGlyProThrSer HisAspCysIleTyrTyrProTppThrGly HisSerThrLeuProGlnHisAlaArg
 1891 AAC TGCACCCCAAGCGGTGAACCGTCCCACATAGT CATGACTGCATTTACTACCCATGGACGGGC CATTCACATTTACCACAAACATGCTAGA
 650 Thr ProLeuIleAlaAlaGlyValIleGlyGly LeuPheIleLeuValIleValGlyLeuThr PheAlaValTyrValArgArgLysSer
 1981 ACT CCGCTGATTGCAGCTGGAGTAATTGGTGGG CTCTTCATTCGTCTGCTCATTTGGGTCTGACA TTGCTGTTTATGTTAGAGGAGAGC
 680 Ile LysLysLysArgAlaLeuArgArgPheLeu GluThrGluLeuValGluProLeuThrPro SerGlyThrAlaProAsnGlnAlaGln
 2071 ATC AAAAAGAAAGAGCCCTTGAGAGATTCTTG GAACAGAGTTGGTGGAACCAATTAACCTCC AGTGGCACAGCACCCCAATCAAGCTCAA

Figure 1

(continued)

710 Leu Arg Ile Leu Lys Glu Thr Glu Leu Lys Arg Vallys Vallys Leu Glu Lys Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Ile Thr Val Pro
 2161 CTT CGTATTTTGAAGAAGAACTGAGCTGAAGAGG GTAAAAGTCTTGCTGCTCAGGTCTTTTGGG ACCGTTTATTAAGGTATTTGGGTACCT
 740 Glu Gly Glu Thr Vallys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro Lys Ala Asn Val Glu Phe Met Asp Glu Ala
 2251 GAA GGAGAAACTGTGAAGATTCCTGTGGCTATT AAGATTTCTTAATGAGACAACTGCTGCCAAG GCMAATGTGGAGTTCATGGATGAAGCT
 770 Leu Ile Met Ala Ser Met Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr Ile Glu Leu Val Thr Glu Leu Met
 2341 CTG ATCATGGCAAGTATGGATCATCCACACCTA GTCCGGTGTGCTGGGTGTGTGTGAGCCCA ACCATCCAGCTGGTACTCAACTTATG
 800 Pro His Gly Cys Ileu Leu Glu Tyr Val His Glu His Lys Asp Asn Ile Gly Ser Glu Leu Leu Leu Asn Thr Cys Val Glu Ile Ala Lys
 2431 CCC CATGGCTGCTGTTGGAGTATGTCACGAG CACAAGGATAACATGGATCACAACTGCTG CTTAACTGGTGTGTCAGATAGTAGTAAG
 830 Gly Met Met Tyr Leu Glu Glu Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Vallys Ser Pro Asn His Vallys Ile
 2521 GGA ATGATGTACTCTGGAAGAAAGCAGACTCGTT CATCGGAATTGGCCAGCCCGTAATGCTTAA GTGAATCTCCAAACCATGTGGAATC
 860 Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Thr Met Ala Leu
 2611 ACA GATTTGGGCTAGCCAGACTCTTGGAGGA GATGAAGAAGAGTACATGCTGATGGAGGA AAGATGCCAATTAAATGATGGCTCTG
 890 Glu Cys Ile His Tyr Arg Lys Phe Thr His Glu Ser Asp Val Thr Ser Tyr Gly Val Thr Ile Tyr Glu Leu Met Thr Phe Gly Gly Lys
 2701 GAG TGTATACATTACAGGAATTCACCCATCAG AGTCAGCTTTGGAGCTATGGAGTTACTATA TGGGAAGTGAATGACCTTTGGAGGAAAA
 920 Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Glu Pro Pro Ile Cys Thr Ile Asp Val
 2791 CCC TATGATGGATTCACACCGCAGAAATCCCT GATTTATTAGAGAAGGAGAGCTTTGGCT CAGCCTCCCATCTGCACATATTGACGTT
 950 Tyr Met Val Met Vallys Cys Tyr Met Ile Asp Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg
 2881 TAC ATGGTCATGCTCAATGTTGGATGATGAT GCTGACAGTAGACCTAAATTTAAGGAAGTGC CTCTCTGAGTTTTCAGAGATGGCTCGA
 980 Asp Pro Glu Arg Tyr Leu Val Ile Glu Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Glu Asn Leu Leu Asp
 2971 GAC CCTCAAGATACCTAGTTATTCAGGGTGAT GATCGATGAGCTTCCAGTCCAAATGAC AGCAGTCTTTTCAGAAATCTCTTGAT
 1010 Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val Pro Glu Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala
 3061 GAA GAGGAATTTGGAGATATGATGGATGCTGAG GAGTACTTGGTCCCTCAGGCTTTCAACATC CCACCTCCCATCTATATCTTCCAGAGCA
 1040 Arg Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly Asn Glu Phe Val Tyr Arg Asp Gly
 3151 AGA ATTGACTCGAATAGGAGTGAATTTGGACAC AGCCCTCTCTCTGCTACACCCCATGTCA GGAACAGTTTGTATATACCCAGATGCA
 1070 Gly Phe Ala Ala Glu Glu Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val Ala Glu Gly Ala Thr Ala
 3241 GGT TTTGCTGCTGAACAAGAGTGTCTGTGCCC TACAGAGCCCCACTAGCACAAATTCAGAA GCTCTGTGGCACAGGGTCTACTGCT
 1100 Glu Ile Phe Asp Asp Ser Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val Glu Glu Asp Ser Ser Thr Glu Arg Tyr Ser
 3331 GAG ATTTTGTGACTCTCTGTTAATGGCACC CTACGCAAGCCAGTGGCCACCCCATGTCCAA GAGGACAGTAGCACCCAGAGGTACAGT

Figure 1
(continued)

81

Figure 2A

350 Ser SerAsnIleAspLysPheIleAsnCysThr LysIleAsnGlyAsnLeuIlePheLeuVal ThrGlyIleHisGlyAspProTyrAsn
 1081 TCC AGTAACATTGACAAATTCATAAATGTACC AAGATCAATGGGAATTTGATCTTTCTAGTC ACTGGTATTCAATGGGACCCCTTACAAAT
 380 Ala IleGluAlaIleAspProGluLysLeuAsn ValPheArgThrValArgGluIleThrGly PheLeuAsnIleGlnSerTyrProPro
 1171 GCA ATTGAAGCCATAGACCCAGAGAACTGAAC GTCTTTCCGACAGTCAGAGAGATAACAGGT TTCTGAAACATACAGTCATNGCCACCA
 410 Asn MetThrAspPheSerValPheSerAsnLeu ValThrIleGlyGlyArgValLeuTyrSer GlyLeuSerLeuLeuIleLeuLysGln
 1261 AAC ATGACTGACTTCAGTGTITTTTCTAACCTG GTGACCAATGGTGGAGAGTACTCTATAGT GGCTGTCTCTTCTTATCTCTCAAGCAA
 440 Gln GlyIleThrSerLeuGlnPheGlnSerLeu LysGluIleSerAlaGlyAsnIleTyrIle ThrAspAsnSerAsnLeuCysTyrTyr
 1351 CAG GGCATCACCTCTCTACAGTTCAGTCCCTG AAGGAATCAGCGCAGGAACATCTATATT ACTGACAAACAGCAACCTGTGTATTAT
 470 His ThrIleAsnTyrThrThrLeuPheSerThr IleAsnGlnArgIleValIleArgAspAsn ArgLysAlaGluAsnCysThrAlaGlu
 1441 CAT ACCATTAACTGGACAACACTCTTCAGCACA ATCAACCAGAGAAATAGTAATCCGGACAAAC AGAAAGCTGAAATTTGACTGCTGAA
 500 Gly MetValCysAsnHisLeuCysSerSerAsp GlyCysTyrGlyProGlyProAspGlnCys LeuSerCysArgArgPheSerArgGly
 1531 GGA ATGGTGTGCAACCATCTGTGTCCAGTGAT GGCTGTGGGACCTGGGCCAGACCAATGT CTGTGTGTCTCGCGCTTCAGTAGAGGA
 530 Arg IleCysIleGluSerCysAsnLeuTyrAsp GlyGluPheArgGluPheGluAsnGlySer IleCysValGluCysAspProGlnCys
 1621 AGG ATCTGCATAGAGTCTTGTAACTCTATGAT GGTGAATTTCCGGAGTTTGAGAAATGGCTCC ATCTGTGTGGAGTGTGACCCCACTGT
 560 Glu LysMetGluAspGlyLeuLeuThrCysHis GlyProGlyProAspAsnCysThrLysCys SerHisPheLysAspGlyProAsnCys
 1711 GAG AAGATGGAAGATGGCTCTCTCACATGCCAT GGACCGGTCTCTGACAACTGTACAAAGTGC TCTCAATTTTAAAGATGGCCCAAACTGT
 590 Val GluLysCysProAspGlyLeuGlnGlyAla AsnSerPheIlePheLysTyrAlaAspPro AspArgGluCysHisProCysHisPr
 1801 GTG GAAAATGTCCAGATGGCTTACAGGGGCA AACAGTTTCATTTTCAAGTATGCTGATCCA GATCGGAGTCCCAACCCATGCCATCCA
 620 Asn CysThrGlnGlyCysAsnGlyProThrSer HisAspCysIleTyrTyrProTyrThrGly HisSerThrLeuProGlnHisAlaArg
 1891 AAC TGCACCCCAAGGTGTAAACGGTCCCCTAGT CATGACTGCATTTTACTACCCATGGACGGGC CATTCCACITTTACCAACATGCTAGA
 650 Thr ProLeuIleAlaAlaGlyValIleGlyGly LeuPheIleLeuValIleValGlyLeuThr PheAlaValTyrValArgArgLysSer
 1981 ACT CCCCTGATTCCAGCTGGAGTAATTGGTGGG CTCITTCATTTCTGGTCAATTTGGGTCTGACA TTGCTGTTTATGTTAGAGGAGAGAC

Figure 2A
(continued)

680 Ile LysLysLysArgAlaLeuArgArgPheLeu GluThrGluLeuValGluProLeuThrPro SerGlyThrAlaProAsnGlnAlaGln
 2071 ATC AAAAGAAAGAGCCTTGAGAGATTCTTG GAAACAGAGTTGCTGGACCACTAACTCCC AGTGGCACAGCACCACCAATCAAGCTCAA
 710 Leu ArgIleLeuLysGluThrGluLeuLysArg ValLysValLeuGlySerGlyAlaPheGly ThrValTyrLysGlyIleIleTyrValPhe
 2161 CTT CGTATTTTCAAAGAACTGAGCTGAGAGG GTAAAGTCCCTGGCTCAGGTCTTTTGA ACCTTTATTAAGTATTTTGGTACCT
 740 Glu GlyGluThrValLysIleProValAlaIle LysIleLeuAsnGluThrThrGlyProLys AlaAsnValGluPheMetAspGluAla
 2251 GAA GGAGAACTGTGAAGATTCTGTGCTATT AAGATCTCTTAATGAGACAACTGCTCCCAAG CCNAATGTGGAGTTTCATGGATGAAGCT
 770 Leu IleMetAlaSerMetAspHisProHisLeu ValArgLeuLeuGlyValCysLeuSerPro ThrIleGlnLeuValThrGlnLeuMet
 2341 CTG ATCATGGCAAGTATGGATCATCCACACTA GTCCGGTTGCTGGGTGTGTCTGAGCCCA ACCATCCAGCTGGTTACTCAACTTATG
 800 Pro HisGlyCysLeuLeuGluTyrValHisGlu HisLysAspAsnIleGlySerGlnLeuLeu LeuAsnTyrCysValGlnIleAlaLys
 2431 CCC CATGGCTGCTGTGGAGTATGTCCACGAG CACAAGGATAACATTGGATCACAACTGCTG CTTAAGTGGTGTGTCTCCAGATAGCTAAG
 830 Gly MetMetTyrLeuGluGluArgLeuVal HisArgAspLeuAlaAlaArgAsnValLeu ValLysSerProAsnHisValLysIle
 2521 CGA ATGATGTACCTCGAAGAAAGACGACTCGT CATCGGATTGGCAGCCCGTAATGTCTTA GTGAATCTCCAAACCATGTGAAATC
 860 Thr AspPheGlyLeuAlaArgLeuLeuGluGly AspGluLysGluTyrAsnAlaAspGlyGly LysMetProIleLysTyrMetAlaLeu
 2611 ACA GATTTTGGCTAGCCAGACTCTTTGGAGGA CATGAAAAGAGTACAAATGCTCATGGAGGA AAGATGCCAATTAATGATGGTCTCTG
 890 Glu CysIleHisTyrArgLysPheThrHisGln SerAspValTyrPsrTyrGlyValThrIle TrrGluLeuMetThrPheGlyGlyLys
 2701 GAG TGTATACATTACAGGAAATTCCACCCATCAG AGTGACGTTTGGAGCTATGGAGTTACTATA TGGGAACGTGATGACCTTTTGAGGAAAA
 920 Pro TyrAspGlyIleProThrArgGluIlePro AspLeuLeuGluLysGlyGluArgLeuPro GlnProProIleCysThrIleAspVal
 2791 CCC TATGATGGAATTCCACGCGGAGAAATCCCT GATTATTAGAGAAAGGAGAACGTTTGCT CAGCCTCCCATCTGCCTACTATTGACGTT
 950 Tyr MetValMetValLysCysTyrMetIleAsp AlaAspSerArgProLysPheLysGluLeu AlaAlaGluPheSerArgMetAlaArg
 2881 TAC ATGGTCATGGTCAAAATGTTGGATGATTGAT GCTGACAGTAGACCTAAATTTAAGGAACTG GCTGCTGAGTTTTCNAGGATGGCTCGA
 980 Asp ProGlnArgTyrLeuValIleGlnGlyAsp AspArgMetLysLeuProSerProAsnAsp SerLysPhePheGlnAsnLeuLeuAsp
 2971 GAC CCTCAAGATACCTAGTTATTTCAGGGTGAT GATCGTATGAAGCTTCCAGTCCAAATGAC AGCAAGTTCTTTTCAGAAATCTCTTGGAT
 1010 Glu GluAspLeuGluAspMetMetAspAlaGlu GluTyrLeuValProGlnAlaPheAsnIle ProProProIleTyrThrSerArgAla
 3061 GAA GAGGATTTGGAAGATATGATGCATGCTGAG GAGTACTTGGTCCCTCAGGCTTTCACACATC CCACCTCCCATCTATCTACTTCCAGAGCA

Figure 2A
(continued)

[illegible]

Figure 2A
(continued)

Figure 2B

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HER4

HER4 with alternate 3'-end without Autophosphorylation domain

```

MKPATGLWVWVSLVAAGTVQPSDSQSVCACTENKLSLSDLQYRALAKYENCCEVVM      60
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
MKPATGLWVWVSLVAAGTVQPSDSQSVCACTENKLSLSDLQYRALAKYENCCEVVM      60

GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAI F   120
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRIIRGTKLYEDRYALAI F   120

LNRYKDGNFGLQELGLQNLTEILNGGVYVDQNKFLCYADTIHQDIVRNPMPSNLTIVST    180
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
LNRYKDGNFGLQELGLQNLTEILNGGVYVDQNKFLCYADTIHQDIVRNPMPSNLTIVST    180

NGSSGCGRCHKSCGTGRMGPTENHQOTLTRTVCAEQCDGRCYGPYVSDOCHRECAGGCSG    240
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
NGSSGCGRCHKSCGTGRMGPTENHQOTLTRTVCAEQCDGRCYGPYVSDOCHRECAGGCSG    240

PKOTDCFACHNFDSGACVTQCPQT FVYNPTTFQLEHNFNAKYTYGAFVCVKCPHNFVVD    300
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PKOTDCFACHNFDSGACVTQCPQT FVYNPTTFQLEHNFNAKYTYGAFVCVKCPHNFVVD    300

SSSCVRACPFSSKMEVEENGIMCKPCTDICPKACDGIGTGSLSAQTVDSNIDKFINCT      360
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
SSSCVRACPFSSKMEVEENGIMCKPCTDICPKACDGIGTGSLSAQTVDSNIDKFINCT      360

KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL    420
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
KINGNLIFLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL    420

VTIGGRVLYSGLSLILKQQGITSLOFQSLKEISAGNIYITDNSNLCYYHTINWTLFST      480
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
VTIGGRVLYSGLSLILKQQGITSLOFQSLKEISAGNIYITDNSNLCYYHTINWTLFST      480

INQRIVIRDNRKAENCTAEGHVCNHLCS SDGCMGPGPDQCLSCRRFSRGRICIESCNLYD    540
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
INQRIVIRDNRKAENCTAEGHVCNHLCS SDGCMGPGPDQCLSCRRFSRGRICIESCNLYD    540

GEFREFEINGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHF KDGPNCVEKCPDGLQGA    600
::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
GEFREFEINGSICVECDPQCEKMEDGLLTCHGPGPDNCTKCSHF KDGPNCVEKCPDGLQGA    600

```

Figure 3A

NSFI FKYADPDRECHPCHPNCTQGCGPTSHDCIYYFWTGHSTLPQHARTPLIAAGVIGG	660
NSFI FKYADPDRECHPCHPNCTQGCGPTSHDCIYYFWTGHSTLPQHARTPLIAAGVIGG	660
LFILVI VGLTFAYVVRKSIKKKRALRRFLETTELVEPLTPSGTAPNQAQLRIKTELKR	720
LFILVI VGLTFAYVVRKSIKKKRALRRFLETTELVEPLTPSGTAPNQAQLRIKTELKR	720
VKVLGSGAGFTVYKGIWVPEGETVKI PVAIKILNETTGPKANVEFMDEALIMASHDHPHL	780
VKVLGSGAGFTVYKGIWVPEGETVKI PVAIKILNETTGPKANVEFMDEALIMASHDHPHL	780
VRLGVCLSPTIQLVTQLMHPHGCLLEYVHEHKDNIGSOLLNMCVQIANGMYLEERRLV	840
VRLGVCLSPTIQLVTQLMHPHGCLLEYVHEHKDNIGSOLLNMCVQIANGMYLEERRLV	840
HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPKMALECIHYRKFTHQ	900
HRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGKMPKMALECIHYRKFTHQ	900
SDVMSYGVTIWELMTFGGKPYDGIPTREIPDLEKGERLPQPPICTIDVYVMVVKWMID	960
SDVMSYGVTIWELMTFGGKPYDGIPTREIPDLEKGERLPQPPICTIDVYVMVVKWMID	960
ADSRPKFKELAAEFMRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLEEDLEDMDAE	1020
ADSRPKFKELAAEFMRMARDPQRYLVIQGDDRMKLPSPNDSKFFQNLLEEDLEDMDAE	1020
EYLVPOAFNI PPPIYTSRARIDSNRSEIGHSPPPAYTPMSGNQFVYRDGGFAAEQGVSVF	1080
EYLVPOAFNI PPPIYTSRARIDSNRVRNNYIHIS-YSF	1057
YRAPSTIPEAPVAQGATAEIFDDSCNGTLRKPVAPHVQEDSSTQRYSDPTVFAPERS	1140
PRGELDEEGYMTPMRDKPKQEYLNPEENPFVSRKNGDLQALONPEYHNASNGPPKAED	1200
EYVNEPLYLNTFANTLGKAEYLNKNNILSMPEKAKKAFDNPDYNNHSLPPRSTLQHPDYLO	1260
EYSTKYFYKQNGRIRPIVAENPEYLSEFSLKPGTVLPPPPYRHRNTVV	1308

Aligned 1058, Matches 1046, Mismatches 12, Score 132, Homology 98%

Figure 3A
(continued)

HER4 N-terminal truncated with autophosphorylation domain

60
120
180
240
300
360
420
480
540
600
660

780

13

840

73

900

133

960

193

1020

253

1080

313

1140

373

1200

433

1260

493

1308

541

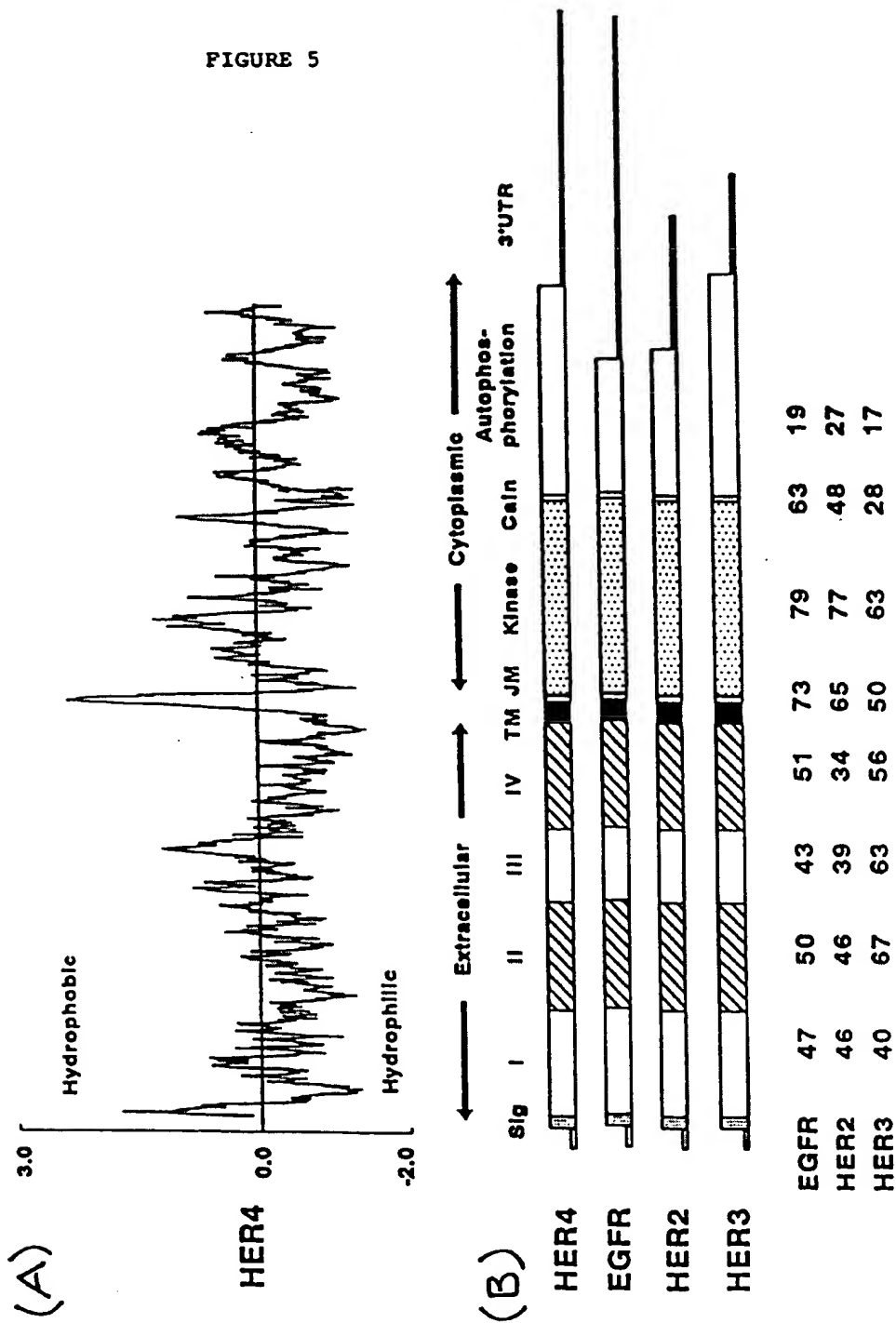
Aligned 541, Matches 541, Mismatches 0, Score 130, Homology 100%

[illegible]

Figure 4

92

FIGURE 5



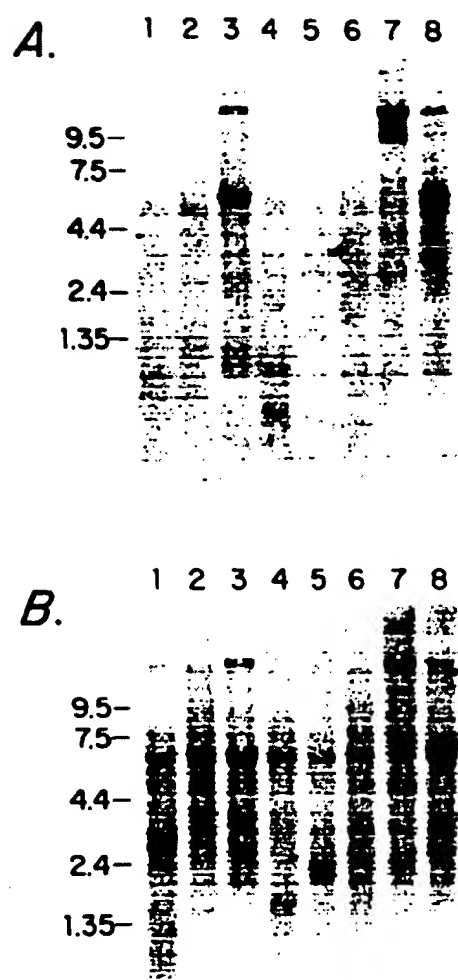


FIGURE 6

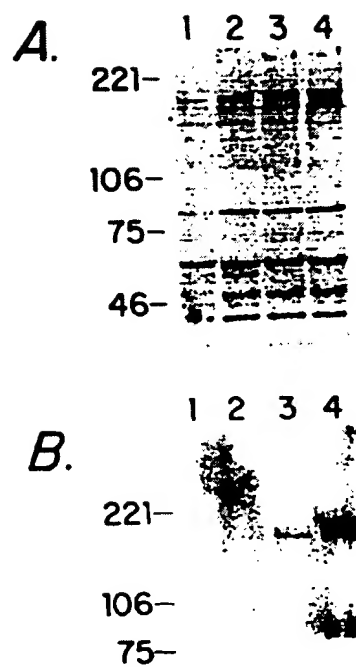


FIGURE 7

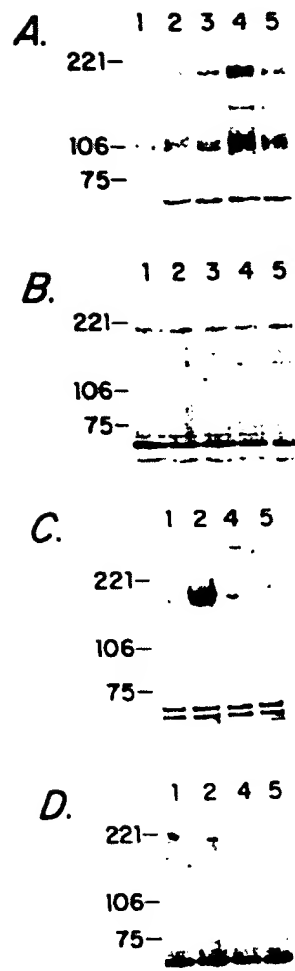


FIGURE 8

Biological and Biochemical Properties of
the MDA-MB-453-cell Differentiation Activity

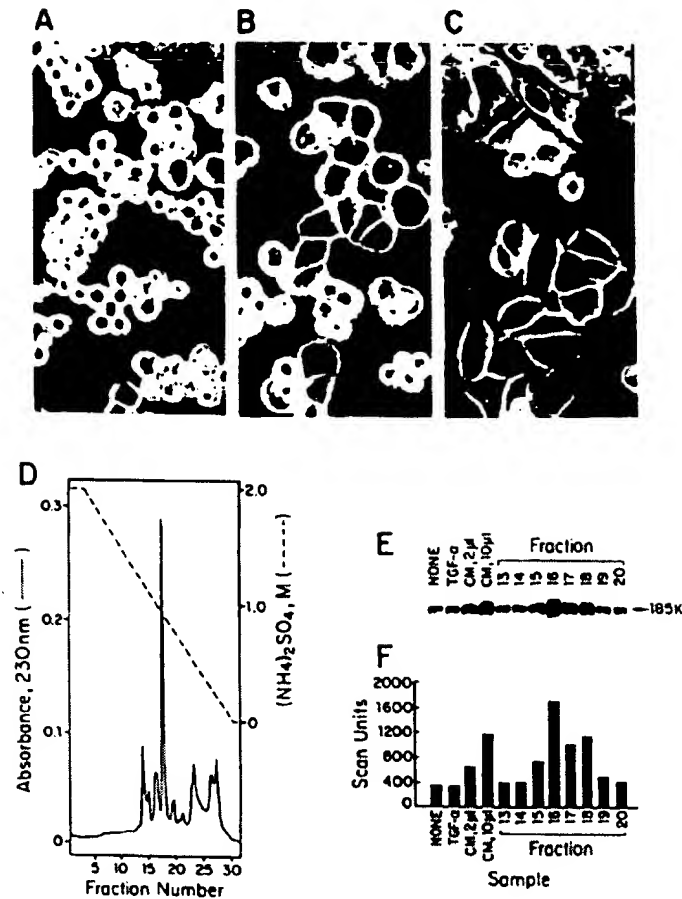


FIGURE 9



FIGURE 10A

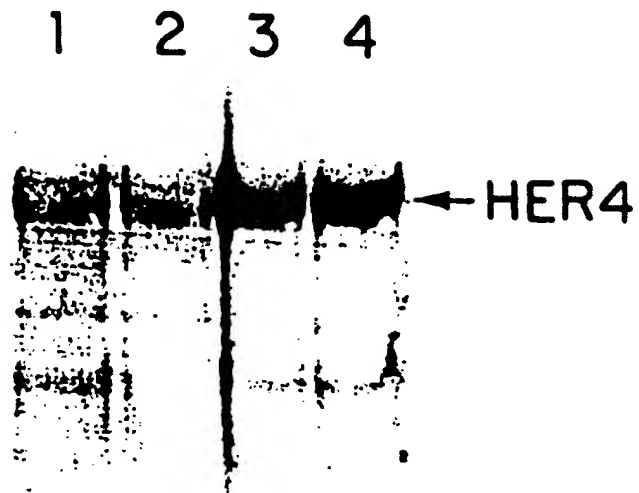
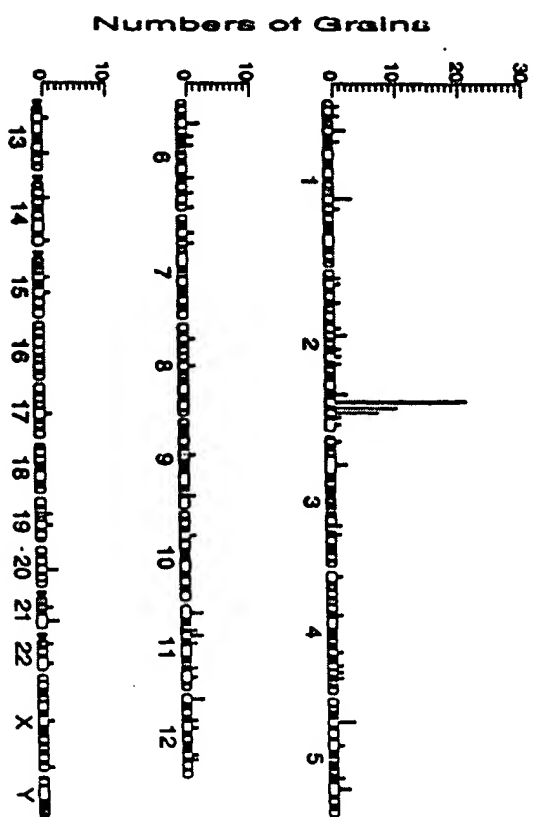


FIGURE 10B

a



H4

b

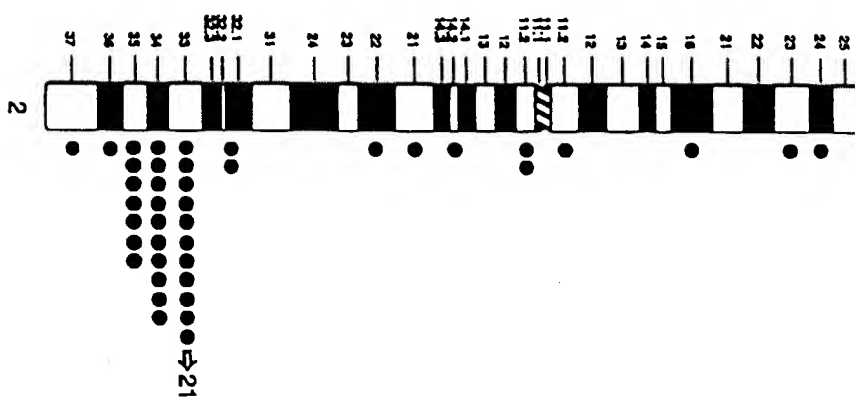


Figure 11

HER4-Ig

HER4 extracellular domain-human Ig fusion construct

MKPATGLWVWVSLVAAGTVQPSDSQSVCACTENKLSSLSDLQQYRALRKYYENCEVVM
 GNLEITSIEHNRDLSFLRSVREVTGYVLVALNQFRYLPLENLRRIIRGTKLYEDRYALAI
 LNYRKDGNFGLQELGLKNTLTIINGGVYVDQNKFLCYADTIHWQDIVRNPWPSNLT
 NGSSGGRCHKSCTGRCWGPTENHCQTLTRTVCAEQCDGRCYGPYVSDCCHRECAGGCSG
 PKDTCFACMNFNDSGACVTQCPQTFVYNPTTFQLEHNFNAKYTYGAFVCVKKCPHNFVVD
 SSSCVRACPSKMEVEENGIKCKPCTDICPKACDGIGTGSLSAQTVDSNIDKFINCT
 KINGNLI FLVTGIHGDPYNAIEAIDPEKLNVFRTVREITGFLNIQSWPPNMTDFSVFSNL
 VTIGGRVLYSGLSLLILKQQGITSLSLQFQSLKEISAGNIYITDNSNLCYYHTINWTTLFST
 INQRIVIRDNRKAENCTAEGMVCNHLCS SDGCWGPDPDQCLSCRRFSRGRICIESCNLYD
 GEFREFENGSI CVECDPQCEKMEDGLLTCHGPGPDNCTKCSHF KDGPNCVEKCPDGLQGA
 NSFIFKYADPDRECHPCHPNTQGCNGPTSHDCIYYPWTGHSTLPQDPVKVKALEGFPRL
 VGPDDFFGCAEPANTFLDPEEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
 PEVTCVVDVSHEDPEVKFNWYVDGVEVHVAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
 KEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
 LAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHY
 TQKSLSLSPGK

Bold = Signal Sequence

= Immunoglobulin domain

Lower case = HER4 ECD

Figure 12



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 43 of the European Patent Convention EP 93 11 8837
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 8)
P, X	WO-A-92 20798 (GENENTECH, US) 26 November 1992 * Abstract, claims * ---	14, 15, 28, 29	C12N15/12 C07K13/00 C12P21/08 C12N5/10 G01N33/68 G01N33/577 A61K39/395
P, X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 90, 1 March 1993, WASHINGTON US pages 1746 - 1750 PLOWMAN GD; CULOUSCOU JM; WHITNEY GS; GREEN JM; CARLTON GW; FOY L; NEUBAUER MG; SHOYAB M; 'Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family.' * the whole document * ---	1-32	
A	SCIENCE vol. 256, 22 May 1992, LANCASTER, PA pages 1205 - 1210 HOLMES, W.E. ET AL.; 'Identification of heregulin, a specific activator of p185erbB2' * the whole document * --- -/--	14, 15	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 8)
			C12N C07K G01N
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely:</p> <p>Claims searched incompletely:</p> <p>Claims not searched:</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		9 March 1994	Nauche, S
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, not published on, or after the filing date D : document cited in the application L : document cited for other reasons a : member of the same patent family, corresponding document	

EP 93 11 8837



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

EP 93 11 8837

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.CLS)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 268, no. 25 , September 1993 , BALTIMORE US pages 18407 - 18410 CULOUSCOU JM;PLOWMAN GD;CARLTON GW;GREEN JM;SHOYAB M; 'Characterization of a breast cancer cell differentiation factor that specifically activates the HER4/p180erbB4 receptor.' * page 18410, column 1, line 4 - page 18410, column 2, line 2 * ---	14,15	
A	EP-A-0 444 961 (BRISTOL-MYERS SQUIBB COMPANY) 4 September 1991 * the whole document * ---	1-32	TECHNICAL FIELDS SEARCHED (Int.CLS)
A	WO-A-90 14357 (GENENTECH, US) 29 November 1990 -----		



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SHEET C

EP 93118817.9

Remark : Although claim 32 is directed to a method of treatment of human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.